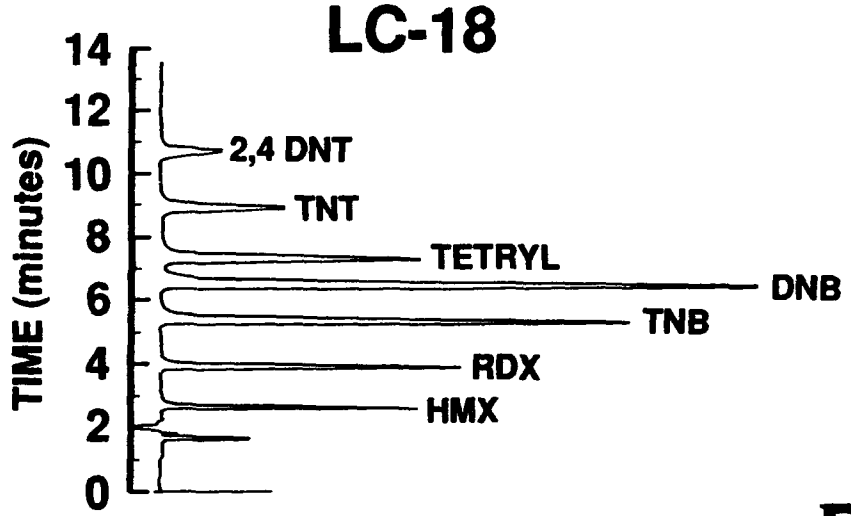
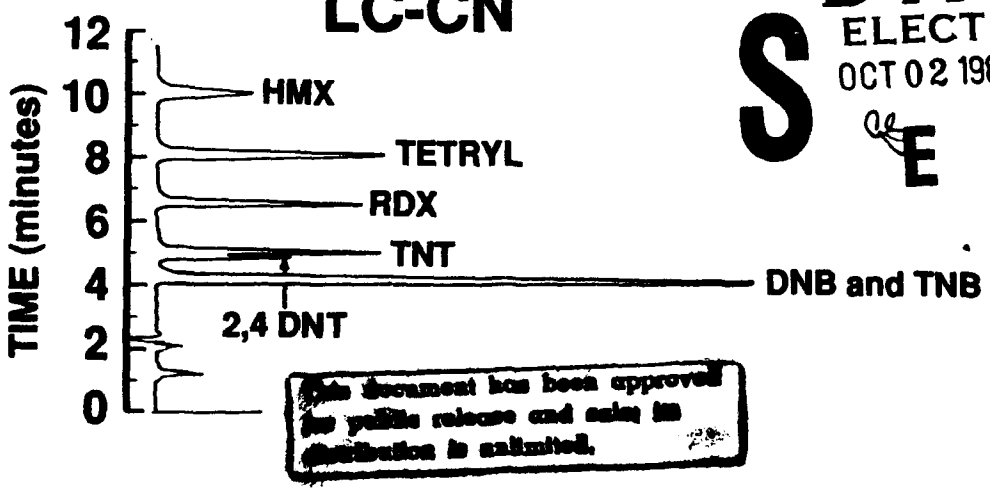
CRREL REPORT 88-8

US Army Corps of Engineers
Cold Regions Research & Engineering Laboratory

Development of an analytical method for the determination of explosive residues in soil

Part II: Additional development and ruggedness testing





For conversion of SI metric units to U.S./British customary units of measurement consult ASTM Standard E380, Metric Practice Guide, *published by the American Society for Testing and Materials, 1916 Race St., Philadelphia, Pa. 19103.*

Cover: Example of the separations achieved for a standard solution on the primary and confirmation columns using the same element (1:1 water-methanol). Note the differences in elution order, particularly for HMX, RDX and TNT.

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July 1988

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Part II: Additional development and ruggedness testing

Thomas F. Jenkins, Patricia W. Schumacher, Marianne E. Walsh
and Christopher F. Bauer

Prepared for
U.S. ARMY TOXIC AND HAZARDOUS MATERIALS AGENCY
REPORT AMXTH-TE-CR-87138

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704 0188
Exp Date Jun 30 1986

1a REPORT SECURITY CLASSIFICATION Unclassified	1b RESTRICTIVE MARKINGS
2a SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.
2b DECLASSIFICATION / DOWNGRADING SCHEDULE	
4 PERFORMING ORGANIZATION REPORT NUMBER(S) CRREL Report 88-8	5 MONITORING ORGANIZATION REPORT NUMBER(S) AMXTH-TE-CR-87138
6a NAME OF PERFORMING ORGANIZATION U.S. Army Cold Regions Research and Engineering Laboratory 6b OFFICE SYMBOL (If applicable) CECRL	7a NAME OF MONITORING ORGANIZATION U.S. Army Toxic and Hazardous Materials Agency
6c ADDRESS (City, State, and ZIP Code) Hanover, New Hampshire 03755-1290	7b ADDRESS (City, State, and ZIP Code) Aberdeen Proving Ground, Maryland
8a NAME OF FUNDING / SPONSORING ORGANIZATION 8b OFFICE SYMBOL (If applicable)	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c ADDRESS (City, State, and ZIP Code)	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO / PROJECT NO / TASK NO / WORK UNIT ACCESSION NO

11 TITLE (Include Security Classification)
Development of an Analytical Method for the Determination of Explosive Residues in Soil
Part II: Additional Development and Ruggedness Testing

12 PERSONAL AUTHOR(S)
Jenkins, Thomas F.; Schumacher, Patricia W.; Walsh, Marianne E.; and Bauer, Christopher F.

13a TYPE OF REPORT	13b TIME COVERED FROM ____ TO ____	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
		July 1988	57

16 SUPPLEMENTARY NOTATION

17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Chemical analysis; Soils; Explosives; Soil contamination

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The analytical method for determination of explosive residues in soil developed by Jenkins and Walsh (1987) was tested and modified to improve its usability. The major modification is the use of an aqueous $CaCl_2$ solution to achieve flocculation and settling of suspended particulates prior to filtration. Ruggedness testing demonstrated that the method is not sensitive to minor modifications in analytical protocol. Specific studies indicated that the following had negligible effects on determined soil concentrations: the degree of grinding prior to extraction with acetonitrile, the ratio of soil mass to extraction solvent volume, the kind of mixing (vortex mixing or manual shaking) used prior to ultrasonic bath extraction, the concentrations of $CaCl_2$ used for flocculation, the length of time allowed after flocculation before samples were filtered, and the number of samples processed simultaneously in the ultrasonic bath. Specific studies were conducted to determine how long stock and working standards and soil extracts were stable. The combined analyte stock solution is good for at least a year, and the combined working standard is good for at least 28 days. Results indicated that soil extracts can be held for at least two months before being analyzed without measurable analyte loss. Care needs to be taken to ensure that air drying

20 DISTRIBUTION / AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	Unclassified
22a NAME OF RESPONSIBLE INDIVIDUAL Thomas F. Jenkins	22b TELEPHONE (Include Area Code) 603-646-4100 22c OFFICE SYMBOL CECRL-RC

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted
All other editions are obsolete

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

19. Abstract (cont'd)

is not conducted in direct sunlight; otherwise losses of TNT will result. The authors recommend a full collaborative test of the method to define performance characteristics in everyday use.

PREFACE

This report was prepared by Thomas F. Jenkins, Research Chemist, and Patricia W. Schumacher, Physical Sciences Technician, both of the Geochemical Sciences Branch, Research Division; Marianne E. Walsh of the Applied Research Branch, Experimental Engineering Division, U.S. Army Cold Regions Research and Engineering Laboratory; and Dr. Christopher F. Bauer, Department of Chemistry, University of New Hampshire.

Funding for this research was provided by the U.S. Army Toxic and Hazardous Materials Agency, Aberdeen Proving Ground, Maryland (R-90 Multi-Analytical Services), Martin H. Stutz, Project Monitor.

The authors acknowledge Dr. Clarence L. Grant, Chemistry Department, University of New Hampshire, and Daniel C. Leggett of CRREL for their technical review of this report. The authors also acknowledge the technical assistance of Elizabeth Rose and Paul Miyares in conducting some of the experiments described and Dean Pidgeon for his assistance in data reduction and manipulation. They also thank Dr. Bruce Tomkins of Oak Ridge National Laboratory for useful comments on the method and Dee Cardin of the University of New Hampshire and Clifford Baker of Wilson Laboratories, Salina, Kansas, for testing the method using soil samples supplied by CRREL.

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ABBREVIATIONS

HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
TNB	1,3,5-trinitrobenzene
DNB	1,3-dinitrobenzene
Tetryl	methyl-2,4,6-trinitrophenylnitramine
TNT	2,4,6-trinitrotoluene
2,4-DNT	2,4-dinitrotoluene
2-Am-DNT	2-amino-4,6-dinitrotoluene
4-Am-DNT	4-amino-2,6-dinitrotoluene
USATHAMA	U.S. Army Toxic and Hazardous Materials Agency
SARM	Standard Analytical Reference Material available through USA THAMA, Aberdeen Proving Ground, Md., 21010.

Development of an Analytical Method for the Determination of Explosive Residues in Soil

Part II. Additional Development and Ruggedness Testing

THOMAS F. JENKINS, PATRICIA W. SCHUMACHER, MARIANNE E. WALSH AND CHRISTOPHER F. BAUER

INTRODUCTION

Over the past few years, CRREL has devoted a great deal of effort toward developing and validating methods for determining munitions residues in environmental samples. Jenkins et al. (1984, 1986) reported a reversed-phase high-performance liquid chromatographic (RP-HPLC) method for determining HMX, RDX, TNT and 2,4-DNT in water. The method involved dilution of a water sample with an equal portion of methanol–acetonitrile, filtration through a disposable 0.45-μm filter, and determination on an LC-8 column using a ternary eluent composed of 50% water, 38% methanol and 12% acetonitrile. Reporting limits were estimated at 26, 22, 14 and 10 μg/L for HMX, RDX, TNT and 2,4-DNT, respectively. Interlaboratory precision ranged from 7–10% for the four analytes from the results of a nine-laboratory collaborative test (Bauer et al. 1986). This method has been accepted by the Association of Official Analytical Chemists (AOAC 1986) as the standard method for determination of explosives in wastewater and groundwater.

A study was conducted to assess the losses of a series of explosives when aqueous and mixed aqueous-organic solvents were filtered through various disposal filter membranes (Jenkins et al. 1987, Walsh et al. 1988). The results indicated that a significant loss of analyte occurred when aqueous solutions were filtered through several commercial filters. The loss was greatest for the first portion of filtrate and for slow filtration. The addition of 50% organic solvent before filtration eliminated sorption losses.

Palazzo and Leggett (1986a, b) reported the development of a method for the determination of TNT and its metabolites in plant tissue. Plant material was extracted by equilibrating fresh tissue with benzene overnight. This was repeated with two fresh portions of benzene; the extracts were combined and diluted to volume. The extract was dried over anhydrous sodium sulfate, and analytes were determined by gas chromatography with an electron capture detector. The concentrations of free TNT and metabolites were determined. The detection limits of TNT and two of its metabolites (2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene) were estimated at about 1 mg/kg, with analytical precision (RSD) of about $\pm$25%. Bound residues were obtained in a like manner after the tissue was heated for 90 minutes with 2.5 M sulfuric acid.

Cragin et al. (1985) conducted the initial work on a method to determine explosive residues in soil. They compared various drying techniques with respect to their effect on analyte recovery. Complete recovery of analyte was achieved using freeze-drying. Significant losses were observed when soils were oven-dried at 105°C. From a practical point of view, air drying was found to be acceptable, with analyte recoveries always in excess of 90%.

Cragin et al. (1985) also conducted experiments on the determination of individual explosives in soil extracts. Gas chromatography, normal-phase high-performance liquid chromatography (HPLC) and RP-HPLC were tested. Overall, RP-HPLC was preferred.

Experiments were also conducted to compare various extraction techniques for explosive residues in soil (Jenkins and Leggett 1985, Jenkins and Grant 1987). Techniques compared were Soxhlet, ultrasonic bath, wrist-action shaker and soil–plant homogenizer. Field-contaminated soils were used for comparisons of the various techniques using methanol and acetonitrile as extraction solvents. Overall, acetonitrile and the sonic bath pro-

Table 1. Soils used in method development.

Soil no.	*Description*	*Clay (%)*	*Organic carbon (%)*
Iowa AAP 3	surface of disposal lagoon	52.5	2.25
Iowa AAP 6	surface of ordnance burning area	52.1	0.70
Louisiana AAP 11	sediment from disposal lagoon	—	—
Louisiana AAP 12	soil next to disposal lagoon	—	—
Milan AAP 10	subsurface soil near disposal lagoon	—	—
Milan AAP 13	surface of burning area	—	—
Milan AAP 14	subsurface (4-6 in.) below burning area	—	—
Milan AAP 15	soil near disposal lagoon	—	—
Milan AAP 16	subsurface (4-6 in.) below burning area	—	—
Milan AAP 17	soil near disposal lagoon	—	—
Nebraska D-49-B	from Nebraska Ordnance Plant	—	—
Nebraska D-16	from Nebraska Ordnance Plant	—	—
USATHAMA standard soil	control soil (uncontaminated)	53.6	1.45

cedure were preferred. Studies using fortified soil indicated that recovery of TNT and RDX was complete at levels as low as 2 μg/g.

Additional experimentation was aimed at developing a completely validated method for determining explosive residues in soils. Jenkins and Walsh (1987) reported a method that involved extraction of a 2-g portion of soil with 50 mL of acetonitrile for 18 hr in a sonic bath. Extracts were diluted 1:1 with water and filtered through a 0.5-μm Millex SR disposable filter assembly. Seven analytes were determined using RP-HPLC. An LC-18 column was used with an eluent composed of 50:50 water-methanol. The analytes were detected with a 254-nm UV detector. Confirmation of the analyte identity was recommended using an LC-CN column with a 1:1 water-methanol eluent, which resulted in a very different elution order than that observed for the LC-18 column.

Certified reporting limits for this method, as defined in USATHAMA (1987), were estimated at 1.6, 1.8, 1.5, 0.5, 5.5, 0.8 and 0.8 μg/g, respectively, for HMX, RDX, TNB, DNB, tetryl, TNT and 2,4-DNT. Precision was better than 0.5 μg/g* in the range of homogeneous variance near the detection limits. At higher concentrations the relative standard deviation was better than ±3%. The method was successfully tested with field-contaminated soil from two army ammunition plants. Recovery was found to be greater than 96% for all seven analytes using fortified soils.

*Analytical precision was poorer for tetryl due to slow decomposition in the extraction solvent.

The objective of the research discussed in this report is to complete the method development and establish the sensitivity of the method to subtle changes in the established protocol, prior to conducting a collaborative test. In this way participants in the collaborative test and other analysts using the method can be informed as to which steps are particularly responsive to small deviations in the recommended procedures.

Contributions of other researchers to the development of methods for the analysis of explosives in soil are discussed at length by Jenkins and Walsh (1987), and no significant advances have been reported since this review was completed.

EXPERIMENTAL METHODS

Soils

The soil samples used in method testing were field-contaminated soils from Iowa, Louisiana and Milan Army Ammunition Plants and the Nebraska Ordnance Plant. Soils were air-dried to constant weight at room temperature, ground in a mortar and pestle, and passed through a No. 30 mesh (0.595 mm) sieve. The soils were stored in individual bottles and mixed thoroughly prior to use. Table 1 lists the soil samples used.

Chemicals

All calibration solutions were prepared from Standard Analytical Reference Material (SARM) obtained from the U.S. Army Toxic and Hazardous Materials Agency (USATHAMA), Aberdeen

Proving Ground, Maryland. The standards were dried to constant weight in a vacuum desiccator over anhydrous calcium chloride in the dark.

The acetonitrile used as the soil extractant was ChromAR HPLC grade obtained from Mallinckrodt. The methanol used to prepare the RP-HPLC mobile phase was Baker Analyzed Reagent HPLC Grade. The water used for dilution of sample extracts and in preparation of the RP-HPLC mobile phase was purified by a MilliQ Type I Reagent Water System (Millipore Corporation). The methanol and water were combined 1:1 and vacuum-filtered through a Whatman CF-F microfiber filter to remove particulates and to degas the mobile phase prior to use.

Soil extraction

The following extraction procedure was used except where specific steps were systematically varied to observe their effect on method performance. Where steps were varied, specific changes will be described in the section describing that test. Air-dried 2-g subsamples of soil were weighed into 2.5- by 20-cm screw-cap glass test tubes with Teflon-lined caps. A 50-mL aliquot of acetonitrile was added, and the soil was dispersed on a Vortex mixer for 1 min and placed in an ultrasonic bath (Cole-Parmer Model 8845-60) for 18 hr. A 10.0-mL portion of each extract was combined with 10.0 mL of $CaCl_2$ solution (20 g/L), allowed to stand 15 min to complete flocculation, and filtered through a 0.5-μm Millex SR disposable filter assembly. The first 5 mL of filtrate was discarded, and the next 10 mL was retained for analysis.

RP-HPLC determination

All determinations were conducted on an LC-18 column (Supelco) using a 1:1 methanol–water eluent at a flow rate of 1.5 mL/min. Samples were injected by overfilling a 100-μL sampling loop, and absorbances were measured on a fixed-wavelength 254-nm UV detector. The analyte identities were confirmed on an LC-CN column using the same eluent described for the LC-18 separation. The retention times and capacity factors for the analytes of interest are presented in Table 2. A sample chromatogram for a standard solution is shown in Figure 1.

Preparation of standards

The analytical stock standards were prepared by weighing out approximately 100 mg of each dried SARM to the nearest 0.1 mg, transferring it to individual 250-mL volumetric flasks, and diluting it to volume with acetonitrile. The flask closures were wrapped with Parafilm to retard evaporation, and storage was at 4°C in the dark.

Table 2. Retention times and capacity factors for principal analytes. (From Jenkins and Walsh 1987.)

	Retention time (min)		*Capacity factor* k	
Substance	*LC-18*	*LC-CN*	*LC-18*	*LC-CN*
HMX	2.55	9.87	0.49	3.94
RDX	3.82	6.56	1.23	2.28
TNB	5.16	4.27	2.02	1.14
DNB	6.25	4.27	2.65	1.14
Tetryl	7.04	8.08	3.12	3.04
TNT	8.47	5.11	3.95	1.56
2,4-DNT	10.15	4.94	4.94	1.47

A combined analyte stock standard was prepared by pipetting 5.00 mL of the stock solutions of TNT, TNB, DNB and 2,4-DNT and 10.0 mL of the stock solutions of HMX, RDX and tetryl into a 100-mL volumetric flask. This solution contained about 20,000 μg/L of TNT, TNB, DNB and 2,4-DNT and 40,000 μg/L of HMX, RDX and tetryl.

A single working standard was prepared each day, generally by diluting the combined analyte stock standard 1:10 with acetonitrile. Prior research has indicated that calibration curves for these analytes are linear with non-significant intercepts (Jenkins et al. 1984, Jenkins and Walsh 1987). Thus, periodic analysis of a single standard was found to define adequately the relationship between concentration and detector response. Standards were diluted 1:1 with aqueous $CaCl_2$ solution prior to injection, thereby achieving the same solvent strength as that for soil extracts.

Reporting limits and analytical precision for this method were reported elsewhere (Jenkins and Walsh 1987), and a summary of the results is presented in Table 3. Complete documentation of the overall method for the determination of explosive residues in soil, in USATHAMA (1987) format, is presented in Appendix B.

TESTS AND RESULTS

Use of flocculation

Experience with the method developed by Jenkins and Walsh (1987) indicated that it was convenient in all respects with the exception of filtration of extracts prior to RP-HPLC determination.

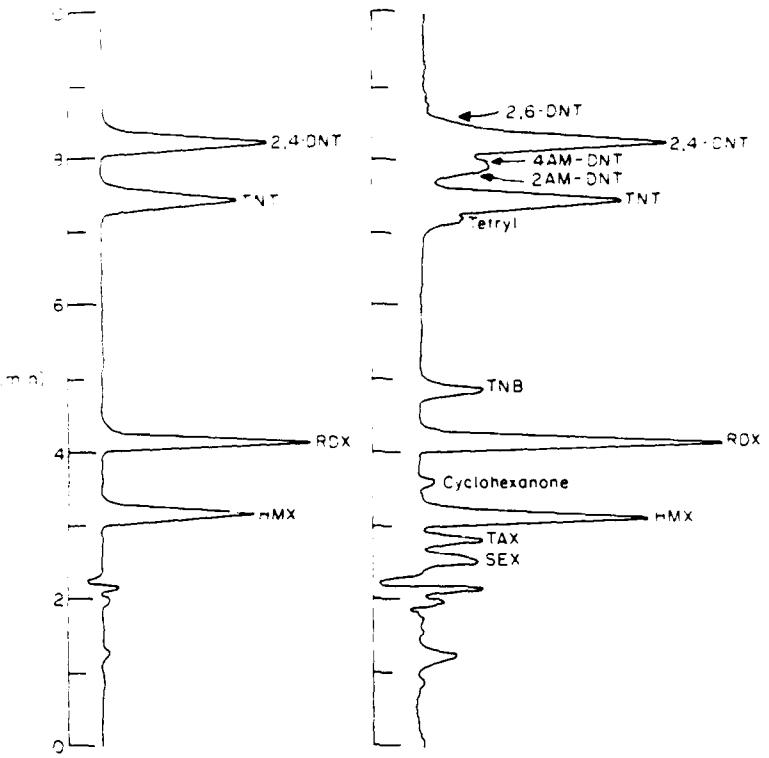


Figure 1. Chromatogram of principal analytes with and without major contaminants on an LC-18 column eluted with 1.5 mL/min 1:1 water-methanol.

Table 3. Reporting limits and analytical precision. (From Jenkins and Walsh 1987.)

Analyte	*Reporting limit* (µg/g)*	*Precision† (µg/g)*
HMX	1.6	0.44
RDX	1.8	0.51
TNB	1.5	0.43
DNB	0.5	0.13
Tetryl	5.5	1.24
TNT	0.8	0.27
2,4-DNT	0.8	0.20

* According to method of Hubaux and Vos (1970) using 2-g soil samples and 50 mL of acetonitrile extractant.

† Obtained from pooled standard deviation over the range of homogeneous variance near the detection limit.

The recommended procedure is to dilute the acetonitrile extract 1:1 with water prior to centrifugation and filtration. Often this produces suspensions of fine clay particles that are difficult to clarify completely by normal centrifugation. When these cloudy supernatants are filtered through 0.5-µm Millex SR filters, the filters plug rapidly, often making it impoasible to obtain sufficient volume for analysis.

One option is to filter the acetonitrile extracts prior to dilution with water. We rejected this option because the solubilities of organic analytes are much reduced in acetonitrile-water compared with pure acetonitrile. Thus if very high concentrations of analyte are present in an extract, small crystals of analyte could precipitate when the extract is diluted with water. If this dilution occurs after filtration, these crystals could be introduced into the sample loop of the HPLC, resulting in severe carryover between samples. Since very high analyte concentrations (% levels) have occasionally been observed in field samples (Jenkins and Walsh 1987), extracts with high analyte concentrations are frequently encountered, and protection against such carryover is a real concern.

A second approach is to dilute with water as described and centrifuge at higher speeds for longer periods of time. Our experience indicates that this requires unbreakable, solvent-resistant centrifuge tubes that also seal sufficiently to inhibit evapora-

tive loss of solvent. Centrifugation is also time consuming, especially when analytical lots of twenty or more samples are processed.

A third approach is to add a flocculating agent such as aqueous $CaCl_2$ solution to the acetonitrile extracts prior to filtration. The major questions regarding this procedure are its effectiveness in removing suspended particulates prior to filtration and the effect this might have on analyte concentrations due to selective adsorption or rejection of analyte by the floc.

In initial flocculation tests an acetonitrile extract from Louisiana 11 soil was mixed 1:1 with a series of 11 aqueous $CaCl_2$ solutions ranging in concentration from 0.01 to 80 g/L. All solutions were shaken and allowed to stand undisturbed for 30 minutes at room temperature. For the two highest $CaCl_2$ concentrations (60 and 80 g/L), two layers formed due to salting out of acetonitrile. For the 0.01-g/L solution, flocculation was not effective. With solutions ranging from 0.1 to 40 g/L, only one liquid layer was visible at room temperature, and flocculation produced complete settling of the floc within 15 minutes. The rate of flocculation and settling appeared to be a function of $CaCl_2$ concentration, with higher concentration solutions settling more rapidly than lower concentrations. Additionally, when solutions prepared by mixing acetonitrile with aqueous $CaCl_2$ solutions with concentrations in excess of 20 g/L were cooled in the refrigerator overnight, two layers formed. This salting-out effect was not observed when the $CaCl_2$ concentration was 10 g/L or less. This result was obtained after a number of tests were conducted with higher $CaCl_2$ levels, but no salting out was observed in these experiments when solutions were maintained at room temperature. From these results, we recommend a $CaCl_2$ concentration of 10 g/L for achieving flocculations and settling of particulates prior to filtrations. To be safe we also recommend that filtered samples be mixed prior to analysis if they have been refrigerated.

To test whether this flocculation technique affected analyte concentrations in extracts, an initial experiment was conducted utilizing a series of eight soils. The explosives were extracted as usual. Two 10-mL aliquots of each extract were placed in separate scintillation vials. A 10-mL portion of water was added to one subsample, and the solution was centrifuged at 2000 rpm for 15 minutes and filtered through a 0.5-μm Millex SR filter as recommended by Jenkins and Walsh (1987). To the second subsample, a 10-mL portion of a 40-g/L $CaCl_2$ solution was added, the solution was allowed to stand for 30 minutes, and the supernatant was filtered through a 0.5-μm Millex SR filter. Each subsample to which $CaCl_2$ was added formed a visible floc that settled rapidly to the bottom of the vial. The resulting supernatants were remarkably clear, while the subsamples that were centrifuged were turbid even after extensive centrifugation. When filtration was conducted, the samples flocculated with $CaCl_2$ filtered very easily, while subsamples that were mixed with water and centrifuged were extremely difficult to filter. Often so much pressure was required to pass liquid through the filters that the holder ruptured and the sample was lost.

Table 4. Mean and standard deviation for ratio of concentrations for centrifuged to flocculated subsamples of extract from eight field-contaminated soils.

	*Ratio (centrifuged/flocculated)**	
Analyte	*Mean*	*Standard deviation†*
HMX	1.00	0.13
RDX	0.98	0.03
TNB	1.00	0.14
TNT	1.00	0.23

* Experimental data in Appendix Tables A1-A4.

† Standard deviation of individual ratios from single determinations for eight soils.

The filtered solutions of all subsamples were analyzed as usual for explosives. The experimental data are presented in Appendix Tables A1-A4 for HMX, RDX, TNB and TNT. A summary of the mean ratios of the analyte concentration in centrifuged subsamples over the analyte concentration in flocculated subsamples is presented in Table 4. These mean values were very close to 1.0 for all four analytes, indicating that the analytical results were nearly equivalent for these two sample preparation methods.

While the results of this initial experiment were encouraging, no analytical replication was used, so it was impossible to determine whether the small differences between centrifuged and flocculated treatments for each individual soil were statistically significant relative to analytical variability. To further pursue the question, three of these soils were selected for an additional study (Iowa 6,

Table 5. Comparison of centrifugation (C) and flocculation (F) procedures with determinations conducted in quadruplicate.

	Concentration (μg/g)											
	HMX		RDX		TNB		DNB		Tetryl		TNT	
Replicate	F	C	F	C	F	C	F	C	F	C	F	C
Milan 13												
1	72.3	70.5	437	437	1.6	2.1	0.81	0.88	34.5	34.0	27.4	27.7
2	70.0	71.7	434	436	2.3	1.9	0.58	0.58	33.4	34.1	27.3	27.8
3	71.5	71.6	448	437	2.0	2.0	1.12	0.73	35.6	33.9	28.0	27.3
4	70.8	70.4	436	435	1.7	2.5	0.93	0.90	35.2	34.7	29.4	28.7
$\bar{X}$	71.2	71.1	439	436	1.9	2.1	0.86	0.77	34.7	34.2	28.0	27.9
S	0.98	0.70	6.3	0.96	0.32	0.26	0.23	0.15	0.96	0.36	0.97	0.59
	t = 0.17*		t = 0.79*		t = 1.09*		t = 0.65*		t = 0.97*		t = 0.26*	
Milan 16												
1	23.8	22.7	172	173	5.3	3.4	1.7	1.4	<d	<d	10.2	9.8
2	23.3	23.7	170	172	4.8	5.0	1.3	1.0	<d	<d	10.5	10.2
3	21.8	23.4	170	172	4.0	4.9	1.6	1.2	<d	<d	10.5	11.0
4	28.0	22.7	171	173	4.8	5.5	1.5	1.3	<d	<d	9.9	11.3
$\bar{X}$	24.2	23.1	171	173	4.7	4.7	1.5	1.2	—	—	10.3	10.6
S	2.7	0.51	0.96	0.58	0.54	0.91	0.17	0.17	—	—	0.29	0.69
	t = 0.81*		t = 3.13*		t = 0.05*		t = 2.48*				t = 0.80*	
Iowa 6												
1	115	118	83.3	78.3	65.5	80.8	<d	<d	<d	<d	757	756
2	117	117	79.1	80.6	65.9	82.3	<d	<d	<d	<d	756	758
3	117	118	79.1	79.6	67.5	83.1	<d	<d	<d	<d	755	756
4	116	120	81.0	80.1	68.0	84.7	<d	<d	<d	<d	748	756
$\bar{X}$	116	118	80.6	79.7	66.7	82.7	—	—	—	—	754	757
S	0.96	1.26	2.0	0.99	1.21	1.63	—	—	—	—	4.1	1.0
	t = 2.53*		t = 0.88*		t = 15.8*						t = 1.19*	

* Critical value for $t_{0.95}(df = 6) = 2.447$.

Milan 13 and Milan 16). Two of these soils were among those with the largest difference between the two types of processing in the initial study. A 2-g subsample of each was extracted as usual, and 10-mL aliquots of each extract were processed by each of the two procedures. Centrifugation was conducted at 5000 rpm for 20 minutes. A 40-g/L aqueous solution of $CaCl_2$ was used for flocculation. The solutions resulting from the two treatments for each soil were analyzed in quadruplicate by the usual procedure. The results are presented in Table 5.

In 4 of the 15 analyte-method comparisons that could be made, mean values for the two treatments were found to be significantly different at the 95% confidence level. For two of these cases (RDX in Milan 16 and HMX in Iowa 6) the percentage difference was 1.2% and 1.7%, respectively. From a practical point of view these differences are unimportant compared to the known variability of analytes in soils. These small differences are statistically significant because of the excellent analytical precision (RSD < 1%).

The concentrations of DNB in the two treatments for Milan 16 were also significantly different at the 95% confidence level but just barely (t = 2.48 compared to a table value of 2.447). Concentrations of DNB for this soil were very low (1.5 and 1.2 μg/g), and the significance is again because the analytical precision was excellent (s = 0.17 μg/g), particularly for such low concentrations.

The fourth statistically significant difference was TNB for Iowa 6. The mean values were 66.7 and 82.7 μg/g for flocculated and centrifuged aliquots, respectively, a difference of 24%. Analytical replication was excellent in both cases, sc the difference appears both real and important. Chromatograms for these extracts are presented in Figure 2. Clearly the TNB peak is lower in the flocculated subsample than in the centrifuged one. However, a small, broad peak eluted just ahead of the TNB peak in the flocculated subsample. When the integrated area of this peak is added to the area of the TNB peak for this subsample, the total area is equivalent to the TNB peak area for the centri-

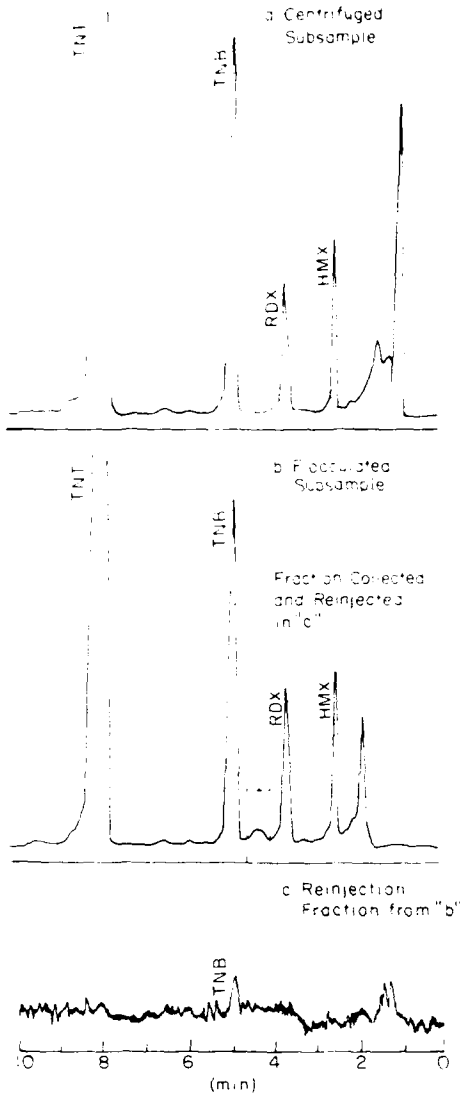


Figure 2. Chromatograms showing extracts of Iowa 6 soil processed by centrifugation and flocculation techniques and reinjection results of broad peak eluting just ahead of TNB in flocculated subsample.

fuged subsample. This observation is consistent with the hypothesis that a portion of the TNB is reacting in some way during flocculation and the product is eluting just ahead of TNB. It is interesting, though, that the absorptivity of this unknown product seems to be equivalent to TNB, since the peak areas add up to that in the centrifuged subsample.

We hypothesized that this small peak eluting ahead of TNB could be due to a complex of TNB and some component released from suspended particles of Iowa 6 after the addition of $CaCl_2$. This complex is not due to a direct interaction of TNB with $CaCl_2$, since the phenomenon was not observed for the other soil extracts discussed earlier, for the TNB standards mixed with aqueous $CaCl_2$ solutions, or for two soil extracts from the Nebraska Ordnance Plant, which had lower but clearly measurable amounts of TNB.

Since the secondary peak elutes ahead of TNB, is broad, and has the same absorptivity as TNB, possibly a charged complex forms initially and then breaks down in the column due to mass action with the eluent, thereby releasing TNB. This would account for premature elution, since a charged complex should move rapidly on a reversed-phase column. The broad nature of the peak may be due to a finite rate of decomposition of the complex on the column. The additivity of peak areas is reasonable since the eluting compound would be uncomplexed TNB itself.

One question remaining is why TNB is complexed but DNB and TNT, which are structurally similar molecules, are not. Space-filling molecular models offer a clue. In TNB the nitro groups may align themselves such that the nitrogens, oxygens and ring carbons are all coplanar, creating an extended pi conjugation system. The electron-withdrawing nature of the nitro groups reduces the electron density on the ring. In TNT the methyl substituent sterically prevents one of the nitro groups from achieving coplanarity, diminishing the degree of electron depletion on the ring. This effect is well known for nitro-substituted aromatics and clearly shows up in UV spectra where the loss of conjugation yields a smaller absorptivity for TNT than for TNB (Dyer 1965). Thus, TNB is unique in that it has a large conjugated pi system with the highest electron density on the perimeter and the lowest in the ring. Such charge separation is conducive to interaction with other species, and its reactivity with electron donors is well established in the literature.

To test this hypothesis, the eluent fraction corresponding to the broad peak eluting ahead of the TNB peak was collected and then reinjected. Figure 2 shows that this material elutes at the same retention time as TNB, which is consistent with the above hypothesis. Further tests were conducted to elucidate why TNB was uniquely affected in the Iowa 6 soil extract by aqueous $CaCl_2$. The occurrence and extent of the problem was independent of $CaCl_2$ concentration; the addition of aqueous $CaCl_2$ to the acetonitrile extract of Iowa 6 always resulted in a lower TNB concentration than that

Table 6. Results of study comparing four alternative treatments for removing particulates from Iowa 6 soil extract.

Concentration (μg/g)	Treatment* A	B	C	D
Initial analysis				
HMX	78	81	77	80
RDX	65	68	67	67
TNB-Complex	15	17	6	21
TNB-Peak	61	62	76	60
Total TNB	86	79	82	81
TNT	731	735	741	734
Analysis conducted after 4-hr standing				
HMX	75	74	77	74
RDX	68	66	71	66
TNB-Complex	6	7	0	7
TNB-Peak	70	70	86	70
Total TNB	76	77	86	77
TNT	735	741	743	741

* *Treatment A*: extract filtered, mixed 50:50 with water.

Treatment B: extract filtered, mixed 50:50 with 10 g/L of aqueous $CaCl_2$.

Treatment C: extract mixed 50:50 with water, centrifuged, filtered.

Treatment D: extract mixed 50:50 with 10 g/L of aqueous $CaCl_2$, allowed to flocculate for 15 min, filtered.

Table 7. Comparison of TNB results for four treatments before and after standing at room temperature overnight.

Concentration (μg/g)	Treatment* A	B	C	D
Initial analysis				
TNB-Complex	16	20	13	17
TNB-Peak	67	64	68	65
Total TNB	83	84	81	82
Analysis conducted after standing overnight				
TNB-Complex	1	1	<d	<d
TNB-Peak	74	73	76	76
Total TNB	75	74	76	76

* *Treatment A*: extract filtered, mixed 50:50 with water.

Treatment B: extract filtered, mixed 50:50 with 10 g/L of aqueous $CaCl_2$.

Treatment C: extract mixed 50:50 with water, centrifuged, filtered.

Treatment D: extract mixed 50:50 with 10 g/L of aqueous $CaCl_2$, allowed to flocculate for 15 min, filtered.

obtained using a procedure involving water addition and centrifugation to remove suspended particulates. The complex appeared to form when water was added as well, as indicated by a small peak eluting just ahead of TNB, but the level was lower than when $CaCl_2$ was added.

In another study, four alternative procedures for removing particulates were tested. Acetonitrile extracts from several replicate 2-g subsamples of Iowa 6 soil were combined, thoroughly mixed, and then divided into two portions. One portion was filtered before any aqueous addition and again split into extracts A and B. Extract A was mixed 1:1 with water,whereas extract B was mixed 1:1 with 10 g/L of aqueous $CaCl_2$. The second, unfiltered portion of the original extract was also divided in half. One portion (extract C) was mixed 1:1 with water, centrifuged at 3000 rpm for 15 minutes, and filtered. Extract D was mixed 1:1 with 10 g/L of $CaCl_2$, allowed to stand 15 minutes for flocculation, and filtered. The solutions resulting from these treatments were analyzed as usual with the added feature that one set of analyses was conducted as soon as possible after sample preparation and a second set of analyses was conducted about 4 hours later (Table 6).

For HMX, RDX and TNT, the four treatments gave equivalent results and there was no significant change in concentration after standing. For TNB, however, immediate analysis of extract C gave high TNB results and low results for the TNB-complex. However, the sum of TNB and TNB-complex was about the same for each of the four treatments. The TNB-complex concentration was reduced after the four-hour waiting period in all cases, and the TNB peak increased. The total TNB for extract C after four hours, however, was somewhat higher than for the other three treatments and for extract C analyzed immediately. Whether this is a real effect or caused by random error is uncertain.

To further explore the effect of allowing the solutions to stand at room temperature prior to analysis, a second study was conducted in an identical manner to the one described above except that after the initial analysis of the four treatments, the solutions were allowed to stand overnight at room temperature before being analyzed again. The results for TNB and the TNB-complex are shown in Table 7. Clearly the TNB-complex was reduced to very low levels for all treatments after the solu-

Table 8. Summary of results for soil-to-solvent ratio test (Appendix Tables A5–A10).

Analyte	Mean concentration (μg/g) 2 g/50 mL		2 g/25 mL		2 g/10 mL		% difference $\frac{\text{highest}}{\text{lowest}} \times 100$
Iowa 3							
HMX	1,990	(a)†	2,000	(a)	1,968	(a)	NS*
RDX	13,580	(b)	13,287	(b)	12,678	(c)	7.1
TNB	484	(d)	479	(d)	474	(d)	NS
DNB	38.4	(e)	38.3	(e)	39.6	(e)	NS
Tetryl	390	(f)	420	(f)	398	(a)	NS
TNT	14,901	(g)	14,764	(g,h)	14,460	(h)	3.0
Louisiana 11							
HMX	224	(i)	228	(i)	264	(j)	17.8
RDX	878	(k)	871	(k,l)	828	(l)	6.0
TNB	1.8	(m)	1.7	(m)	1.7	(m)	NS
DNB	< d		< d		0.15		—
Tetryl	< d		< d		< d		—
TNT	12.2	(n)	12.0	(n)	11.6	(n)	NS

* Difference between three treatments was not significant at the 95% confidence level using ANOVA.

† Numbers identified with the same letter are not significantly different at the 95% confidence level by ANOVA.

tions stood overnight at room temperature, and TNB concentrations showed a coincident increase. However, the total concentration estimates were consistently lower than those reported for immediate analysis.

Soil-to-solvent ratio

The sensitivity of the method's results to variation in the soil-to-solvent ratio was investigated. The method developed by Jenkins and Walsh (1987) specifies 2 g of soil extracted with 50 mL of acetonitrile. For two soils, Iowa AAP 3 and Louisiana AAP 11, 18 replicate 2-g subsamples of each soil were weighed out and randomly divided into three groups of six. One group of six subsamples for each soil type was extracted as usual. The other two groups were extracted with ratios of 2 g to 25 mL and 2 g to 10 mL of solvent, respectively. Extracts were processed and analyzed by the normal procedure. The results of the analytical determinations (Appendix Tables A5–A10) are summarized in Table 8. An analysis of variance was used to compare analyte concentrations. Significant differences were found among the three treatments in only four cases: RDX for both soils, HMX for Louisiana 11 soil and TNT for Iowa 3 soil. The concentrations of TNT and RDX in Iowa 3 soil exceeded 1% of the dry weight of soil, and low recovery was found when the 2-g subsample was extracted with only 10 mL of acetonitrile. Since analyte concentrations in the extract would be five times as great for this treatment compared to the treatment using 50 mL of solvent, it is likely that both chemicals were approaching their solubility limit. Even so, the mean concentrations for the extracts representing 2 g in 10 mL were only 7.1% lower than that for 2 g in 50 mL for RDX and only 3.0% lower for TNT.

For Louisiana 11 soil, which had much lower analyte concentrations, the differences occurred for HMX and RDX in opposite directions. For HMX, 10-mL extracts recovered 17.8% higher concentrations than for 50 mL. For RDX the opposite occurred. The mean concentrations of RDX on a μg/g basis were 6.0% lower for the 10-mL extracts than for the 50-mL extracts. This unusual result for HMX in Louisiana 11 soil is anomalous. The standard deviation associated with 10-mL extracts was four times greater than those for the 25- and 50-mL extracts, and thus the result may be due to poor replication.

The higher solution concentrations achieved for the extracts with 2 g in 10 mL did permit quantitation of DNB for Louisiana 11 soil when it wasn't possible to do so for the 25-mL and 50-mL extracts. This was expected since the reporting limit

determined by Jenkins and Walsh (1987) was 0.5 μg/g for 50-mL extracts, and the value obtained from the 10-mL extracts was 0.15 μg/g.

Overall, the method appears to be quite rugged with respect to the soil-to-solvent ratios tested. This is advantageous in order to extend the range either above or below what can be easily achieved with the 2 g in 50 mL suggested.

Photodegradation study

It is well known that TNT degrades in solution in the presence of sunlight. However, the susceptibility of TNT and other munitions to photodegradation when associated with soil is unknown. In general, soils to be analyzed for explosives are air-dried for periods of at least 24 hours prior to extraction. It is important to know how sensitive these components are to exposure to light during the drying period to assess whether special precautions are necessary to minimize such exposure during drying.

Two soils, Louisiana 12 and Iowa 6, were selected for study based on their previously determined concentrations of TNT. A bulk sample of each was air-dried, ground and sieved under low light conditions, homogenized and divided into two portions. One portion of each soil was spread in a thin layer in aluminum pans and exposed to room light and sunlight for 10 days. The pans were kept on the sill of a south-facing window, ensuring maximum exposure to whatever sunlight was available over the period. Two days were sunny and the other eight days were mostly overcast. Fluorescent lights in the room were left on continuously during the ten days. The pans were shaken several times per day to refresh the soil surface exposed to light.

The second portion of each soil was also spread evenly in aluminum pans but were kept in the dark in the same room as the exposed samples. The residual moisture contents of the soils maintained in the dark and those exposed to room light were found to be equivalent.

After the ten-day exposure, six 2-g subsamples of each soil treatment were extracted and analyzed as usual (Table 9). Statistically significant differences in analyte concentrations for the two treatments at the 95% confidence level were observed for RDX and TNT in Louisiana 12 and for TNB and TNT in Iowa 6. A loss of 8.6% and 10.8% for TNT was observed for the light-exposed subsamples of Louisiana 12 and Iowa 6, respectively. A 5.0% increase in RDX concentration was observed in the light-exposed subsamples for Louisi-

Table 9. Results of photodegradation experiment.

	Concentration (μg/g)									
	HMX		*RDX*		*TNB*		*DNB*		*TNT*	
Replicate	*Dark*	*Light*	*Dark*	*Light*	*Dark*	*Light*	*Dark*	*Light*	*Dark*	*Light*
					Louisiana 12					
1	51.4	51.0	162	174	2.5	2.3	<*d*	<*d*	11.9	10.8
2	53.4	54.2	162	165	2.4	2.5	<*d*	<*d*	11.1	10.7
3	51.6	50.0	158	164	2.5	2.3	<*d*	<*d*	11.6	10.7
4	61.5	58.2	161	175	2.2	2.8	<*d*	<*d*	10.8	10.7
5	55.2	60.3	164	165	2.2	2.3	<*d*	<*d*	11.6	10.1
6	55.9	—	160	—	2.5	—	<*d*	<*d*	12.5	—
$\bar{X}$	54.8	54.7	161	169	2.4	2.4	—	—	11.6	10.6
S	3.7	7.4	2.1	5.4	0.14	0.21	—	—	0.59	0.28
	t = 0.04*		t = 3.23*		t = 0.48*				t = 3.36*	
					Iowa 6					
1	61.1	76.6	71.6	80.5	63.9	73.3	0.76	0.42	712	648
2	46.0	47.3	82.1	125.6	65.3	71.9	0.21	0.72	718	649
3	71.5	69.9	60.5	90.3	67.9	71.8	0.62	0.40	745	666
4	67.2	96.2	80.9	61.7	66.7	65.3	0.62	0.59	740	661
5	52.1	53.7	66.5	81.1	60.0	69.7	0.69	0.62	756	656
6	109.0	52.6	80.2	84.4	67.7	66.3	0.71	0.61	734	649
$\bar{X}$	67.8	66.1	73.6	87.3	65.2	69.7	0.60	0.56	734	655
S	22.3	18.5	8.9	21.1	3.1	3.3	0.20	0.12	16.6	7.5
	t = 0.15*		t = 0.77*		t = 2.46*		t = 0.43*		t = 10.7*	

* Table value for $t_{0.95}(9\ df)$ = 2.26, $t_{0.95}(10\ df)$ = 2.23.

ana 12, and a 6.9% increase in TNB concentration was observed in Iowa 6.

The loss of TNT on exposure to light is consistent with its known susceptibility to photodegradation. The coincident increase in TNB concentration in Iowa 6, where the largest change in TNT concentration was observed, could be due to its formation as a degradation product of TNT. The increase in RDX in the Louisiana 12 soil exposed to light was unexpected. RDX cannot be a degradation product of TNT and is unlikely to come from other potential contaminants, but it might be released from soil organic matter or mineral complexes.

While the loss of TNT due to photodegradation was clearly demonstrated for both soils, the loss averaged only about 10% for conditions in which light exposure was maximized. When air-drying soils, it is therefore recommended that the soils be isolated from direct sunlight and that exposure to room light be minimized as much as possible. Grinding and sieving will generally take place only after the soil is dry, so the surface area actually exposed to light during drying will be much less than in our experiment.

Power dissipation in sonic bath

Dr. Bruce Tomkins of Oak Ridge National Laboratory questioned the dependency of the sonic bath extraction procedure on the number of samples being processed simultaneously. He was concerned that processing a large number of tubes at a time could lessen the efficiency of sonic dispersion.

To investigate this we weighed out eight replicate 2-g subsamples of Iowa 6 soil into test tubes. Four tubes were randomly selected and extracted for 18 hours as usual with no other tubes in the bath. The remaining four tubes were processed in an identical manner except that 32 additional tubes were processed simultaneously.

After extraction both sets of replicates were processed and analyzed as usual (Table 10). No significant differences were found between the two treatments at the 95% confidence level for any of the analytes. For TNB and TNT the RSD averaged 2.1%, so the ability to observe a difference between treatments if one was present was powerful. For HMX and RDX, analytical precision was poorer, so the ability to observe a difference was also poor. Nevertheless, it does not appear that there is a measurable difference in analyte concentrations whether sonic bath extraction is conducted with a full rack of 36 tubes or as few as 4.

Ruggedness test

To complete the ruggedness testing of this method, we carefully scrutinized the individual analytical steps and identified four factors that could potentially affect performance and might be varied by individual analysts. These factors were varied systematically by way of a full factorial experiment to assess just how sensitive the method was to each variable or the interaction of several. To conduct a full 2^4 factorial experiment in duplicate throughout requires 32 trials, which is about the maximum number of analyses that can be conducted in one eight-hour day. Conducting all analyses in one day eliminates variability resulting from differences in daily calibration curves.

One important factor was particle size. The method of Jenkins and Walsh (1987) specifies grinding the soil sufficiently to pass a 30-mesh sieve. We questioned whether further grinding to pass a 60-mesh sieve would alter analyte recovery.

The second factor identified was vortex mixing prior to extraction. We felt that some analysts might choose to eliminate this step in favor of

Table 10. Results of sonic power study (Iowa 6 soil).

	Concentration (μg/g)									
	HMX		*RDX*		*TNB*		*DNB*		*TNT*	
Replicate	*four in rack*	*full rack*	*four in rack*	*full rack*	*four in rack*	*full rack*	*four in rack*	*full rack*	*four in rack*	*full rack*
1	77.2	99.7	66.8	94.0	61.7	59.4	0.53	0.48	735	750
2	48.8	85.1	100.6	99.1	60.7	60.5	0.60	0.63	754	751
3	62.8	150.4	74.8	77.3	59.7	60.2	0.65	0.57	748	769
4	49.8	64.6	85.6	58.0	62.9	59.8	0.58	0.40	806	753
$\bar{X}$	59.7	100.0	82.0	82.1	61.3	60.0	0.59	0.52	761	756
S	13.1	36.6	14.6	18.6	1.4	0.48	0.05	0.10	31	8.9
	t = 2.07*		t = 0.01*		t = 1.76*		t = 1.24*		t = 0.31*	

* $t_{0.95}(df = 6) = 2.45$.

Table 11. Design and interaction matrix and the factor levels employed in the 2^4 factorial ruggedness test.

	Variable			
Run*	1	2	3	4
1	–	–	–	–
2	+	–	–	–
3	–	+	–	–
4	+	+	–	–
5	–	–	+	–
6	+	–	+	–
7	–	+	+	–
8	+	+	+	–
9	–	–	–	+
10	+	–	–	+
11	–	+	–	+
12	+	+	–	+
13	–	–	+	+
14	+	–	+	+
15	–	+	+	+
16	+	+	+	+

* All trials run twice and randomly sequenced.

Factors	(+)	(–)
1 particle size	60 mesh	30 mesh
2 agitation	manual	vortex
3 $CaCl_2$ concentration	4 g/L	20 g/L
4 idle time, post-flocculation	4 hr	0.25 hr

Note: For all factors the (–) level is that specified by the original method.

manual shaking. The levels tested were the normal 1-minute vortex mixing versus 15 seconds of manual shaking.

The third factor chosen was the concentration of the aqueous $CaCl_2$ solution added to the acetonitrile extracts. Previous studies indicated that $CaCl_2$ concentrations could vary over a wide range and still produce flocculation, but possible changes in analyte concentrations were not systematically studied. We chose 20 g/L and 4 g/L as the two levels to be tested. The high level is near the maximum concentration of $CaCl_2$ that can be used without causing the acetonitrile to "salt out" of solution at room temperature, and the low level is still adequate for efficient flocculation.

The final factor identified was idle time, the settling time allowed for flocculation after the $CaCl_2$ solution is added. The two levels chosen were 15 minutes, the minimum time necessary to allow the floc to settle, and 4 hours, both at room temperature.

Two factorial experiments were conducted; one used Iowa 6 soil and the second used Nebraska 49 soil, fraction B. These two soils represented extremes in analyte concentrations, thereby addressing concentration as a possible determining influence in whether these factors significantly affected overall method performance. The design of the 2^4 factor experiments is summarized in Table 11.

Experimentally the combinations specified by the design matrix were obtained as follows. Soil previously ground to pass a 30-mesh sieve was mixed thoroughly and split into two 40-g portions. One portion was further ground to pass a 60-mesh sieve. Sixteen 2-g subsamples of each portion were then weighed into individual 25- × 200-mm test tubes equipped with Teflon-lined screw caps.

A 50-mL aliquot of acetonitrile was added to each tube. Half of the tubes were vortex-mixed for one minute and equilibrated for 18 hours in a sonic bath. The second half of the tubes were manually shaken for 15 seconds and placed in the sonic bath along with the tubes subjected to vortexing.

After sonic bath equilibration, the tubes were allowed to cool, and 5-mL portions of each were removed with a glass volumetric pipette and placed in glass scintillation vials containing 5-mL aliquots of one of the two aqueous $CaCl_2$ solutions. Half the vials contained 20 g/L of $CaCl_2$, while the other half contained 4 g/L of $CaCl_2$. The vials were briefly shaken to mix.

Half of the vials were allowed to stand for 15 minutes, during which the suspended particulates flocculated and precipitated. A 6-mL portion of the clear supernatant was filtered through 0.45-μm Millex SR disposable filters into clean scintillation vials. The first 3-mL portion of each filtrate was discarded, and the second 3-mL portion was retained for analysis. The second half of the extracts were processed as described above except that a 4-hour period of idle time was allowed before filtration. All flocculations occurred at room temperature.

Tables A11 and A12 list the design matrix, random analysis sequence, and results for Iowa 6 and Nebraska D-49-B soils, respectively. All data were checked for transcription errors. Iowa 6 soil values are integrated peak areas, but Nebraska D-49-B values are peak heights. Visual inspection of peak area data (Table A13) for Nebraska D-49-B indicated much greater variability than for peak heights. Since higher variability desensitizes significance tests and could mask some important effects, the ruggedness test results were best anal-

Table 12. Ruggedness test effects for Iowa 6 soil expressed as percent of grand average.

	HMX	*RDX*	*TNB*	*DNB*	*TNT*
s (size)	**15.3***	11.4	0.8	**23.4**	0.5
a (agitation)	-4.8	1.0	-0.6	-15.5	-0.4
c ($CaCl_2$)	**-23.5***	8.0	-0.9	9.3	0.3
i (idle time)	7.0	-4.2	**7.3***	-5.8	-0.1
sa	-2.6	-2.4	-0.3	-0.8	0.2
sc	**20.4***	-0.5	0.9	1.8	-0.1
si	-7.1	-0.1	-0.9	-1.9	-0.1
ac	1.0	-10.8	1.5	-1.6	0.1
ai	**10.9**	-6.6	-0.7	**-23.8**	0.4
ci	**-9.1**	2.8	0.8	-5.3	-0.1
sac	-0.2	-0.3	-0.6	-18.3	-0.8
sai	**-16.7***	5.2	-0.3	3.7	-0.6
sci	4.0	-3.9	0.0	-15.5	0.2
aci	**-9.3**	-2.3	0.8	2.5	-0.2
saci	**15.5***	2.5	-1.2	-7.8	0.0
% rsd	11.6	15.7	3.8	25.4	1.6

Effects in boldface are significant at 95% probability level.
Effects with * are significant at 99% probability level.

yzed by means of peak heights. However, the standard deviation of the peak heights is misleadingly small because the method specifies integration. For this reason, area data were also analyzed solely for the purpose of estimating method variance.

An analysis of variance (ANOVA) was performed separately on each analyte to discover whether any of the four factors had significant effects on analyte recovery. In addition, several diagnostic tests were performed to check for hidden effects (such as time and concentration) and for validity of ANOVA's underlying assumptions (in particular, homogeneity of variance).

Tables A14–A18 contain ANOVA tables for Iowa 6 and Tables A19–A25 for D-49-B soil. Significant effects are identified when the F ratio for an effect exceeds the critical values: $F_{0.95}(1,16) = 4.49$ or $F_{0.99}(1,16) = 8.53$. Each effect has one degree of freedom, and the replication error has 16 degrees of freedom because 16 sets of duplicates were run. The tables generated by the computer software list the t statistic instead of F; in this case $t^2 = F$ (when there is only one degree of freedom in the numerator of the ratio) (Box et al. 1978). Critical t values thus are $t(0.95) = 2.12$ and $t(0.99) = 2.92$.

Tables 12 and 13 summarize all of the effects expressed as the percentage change relative to the grand average. Analyte 2-Am-DNT is listed twice in Table 13—the averages in the first column are based on results that include two concentrations reported as 0.0 μg/g (Table A12); for the second column the zero values were replaced with the grand average of all the 2-Am-DNT data. The original data had a standard deviation much larger than that of other analytes (Table 13). This large standard deviation desensitizes significance tests greatly. The rationale for the replacement is that the zero values are not "normal." Their duplicates (rows 10 and 26 of Table A12) had the values 0.58 and 0.79 μg/g, respectively; hence the zero values occurred inconsistently. Furthermore, in a probability plot of the model residuals* these four runs stood out, meaning the zero values were systematically caused by some uncontrolled factor. Replacing the zeroes with an average (0.59 μg/g) that includes that the zero value attempts to account for these values clearly being low.

Particle size

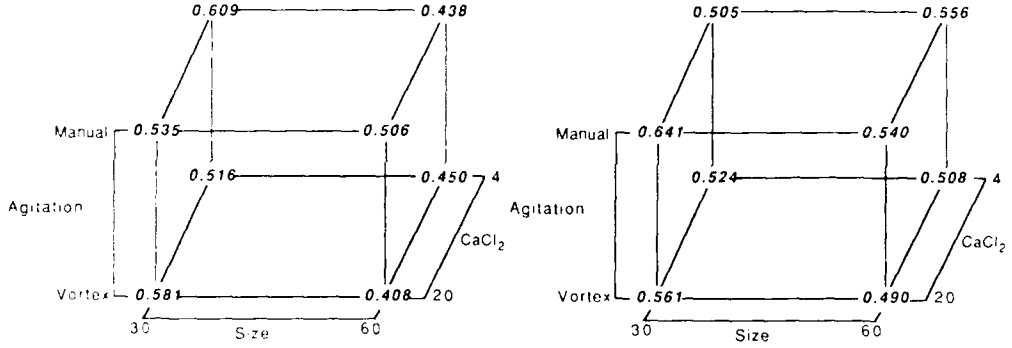
Smaller particles led to significantly higher recoveries for HMX and DNB in Iowa 6 and lower recovery for DNT in D-49-B. Figure 3 is a cube plot for DNT in which the average of each set of duplicates is displayed for every combination of factors. The effect of size on DNT is consistent for every comparison except at four hours of idle time, 4 g/L of salt and manual agitation. The loss-

*The model combines the effects into an algebraic equation that may be used to predict analyte concentration based on the levels of the factors. If the model includes all significant influences on the data, then differences between predicted concentrations and actual concentrations should be randomly distributed around zero and a probability plot should be linear.

Table 13. Ruggedness test effects for Nebraska D-49-B soil expressed as percent of grand average.

	HMX	*RDX*	*TNB*	*TNT*	*DNT*	*2AmDNT*	*2AmDNT*†
s (size)	-4.0	-7.7	-0.9	-1.6	**13.8**	1.0	1.0
a (agitation)	3.7	**-11.0**	-1.2	-7.7	7.0	-14.6	**-13.7***
c ($CaCl_2$)	-1.3	**-14.0**	-3.8	-1.2	- 3.7	19.9	7.0
i (idle time)	-3.1	-0.5	4.2	4.3	6.8	**25.6**	**12.3**
sa	-1.8	-4.8	0.5	1.6	1.9	18.7	5.8
sc	0.8	5.5	2.8	-3.3	4.1	-4.1	-3.8
si	0.0	1.9	0.2	-1.8	7.3	1.9	1.8
ac	0.3	-6.6	-1.5	-0.1	- 1.7	7.4	7.0
ai	0.4	7.6	0.0	8.5	0.6	13.1	**12.3**
ci	**6.7**	8.6	5.4	8.1	- 2.9	-3.1	8.9
sac	1.4	-4.4	2.1	2.3	- 3.7	-15.1	-2.4
sai	0.8	**13.9**	0.5	0.3	- 0.1	1.3	**13.0***
sci	3.9	1.3	1.7	10.3	5.8	-10.0	**-9.4**
aci	1.1	-1.6	2.4	-6.9	- 3.1	10.0	**9.4**
saci	-1.8	-2.9	-0.9	-2.2	8.2	3.4	-8.6
$\sigma_0$rsd	7.4	14.1	7.2	14.4	15.3	31.5	12.2

Effects in boldface are significant at 95% probability level.
Effects with * are significant at 99% probability level.
† Two zero values replaced with grand average of all 2-Am-DNT data.



a. Idle time = 0.25 hr. *b. Idle time = 4 hr.*

Figure 3. Cube plot for concentration (μg/g) of 2,4-DNT in Iowa 6 soil.

es could be due to decomposition through thermal heating during grinding, but DNT is less susceptible to this than are HMX and RDX, which did not show lower recovery. DNT is reliably determined by gas chromatography, which requires its volatilization at high temperatures, whereas HMX and RDX frequently decompose (Jenkins et al. 1984). Another possibility is that the strong adsorption of DNT to a soil component is increased as the surface area increases with the reduction of particle size. DNT is particularly susceptible to adsorption by organic matter. For this suite of analytes it has the greatest octanol-water partition coefficient (Jenkins et al. 1984), elutes most slowly on the reversed phase column, and tends to adsorb to plastic containers (Jenkins et al. 1984) and filters (Jenkins et al. 1986). The size of the effect, however, is small.

Small particles enhance HMX recovery very significantly in Iowa 6 but not in D-49-B, where the concentration is much lower. HMX is apparently more available in Iowa 6 when grinding is more extensive. HMX may be heterogeneously distributed, perhaps as localized deposits or discrete crystals, which may be less efficiently solubilized than a more evenly distributed analyte. Following the original contamination event, water may have evaporated, leading to precipitation of HMX instead of adsorption because of its inherently low solubility (Jenkins et al. 1984) and high concentra-

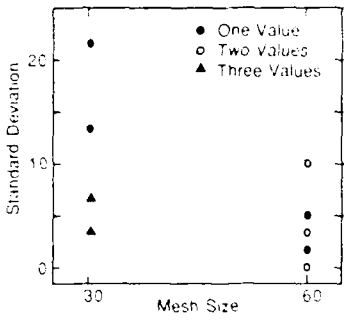
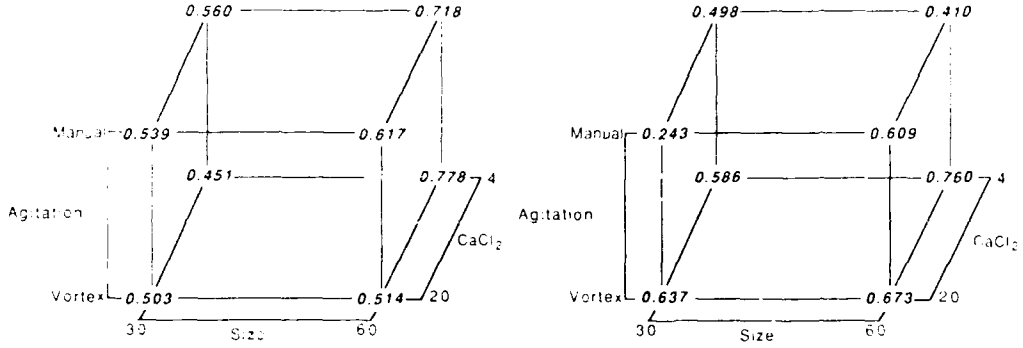


Figure 4. Standard deviations of duplicate measurements for HMX in Iowa 6 soil segregated by particle mesh size.



a. Idle time = 0.25 hr. *b. Idle time = 4 hr.*

Figure 5. Cube plot for concentrations (μg/g) of DNB in Iowa 6 soil.

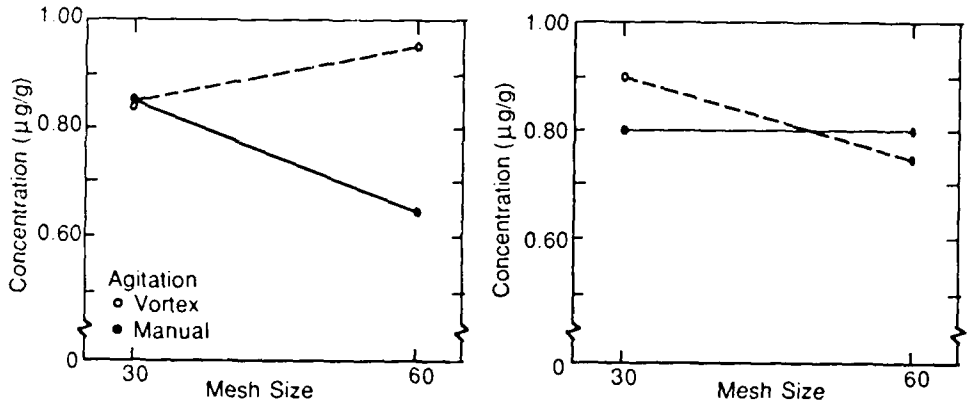
tion in this soil. This interpretation is supported by an earlier study of extraction methods (Jenkins and Grant 1987). Increasing variance with decreasing sample size was found for HMX and RDX in Iowa 6 soil. This trend is consistent with heterogeneous distribution. RDX recoveries in the ruggedness test were 11% higher in Iowa 6 with smaller particles, but the effect was not significant at the 95% level.

An additional confirmation of analyte heterogeneity is that the variance of results for 30-mesh particles was significantly greater than that for 60-mesh particles. Table 14 shows *F* ratios of the variances of each factor between its two levels. The ratio for HMX in Iowa 6 is highly significant. A more appropriate comparison is to look at the variances of individual duplicates as a function of factor level. Figure 4 confirms the variance difference with particle size by segregating HMX by small and large particle size. Similar comparisons were performed for all analytes. The existence of significantly different variances between levels contravenes an assumption in ANOVA of constant variance. The difference would tend to desensitize the significance tests; nevertheless, Table 12 shows that many effects for HMX were significant. The reason for a significant two-factor interaction between size and the level of $CaCl_2$ used is unexplained.

For DNB, larger recoveries for smaller particles (Table 12) is consistent across comparisons (Fig. 5) except for the four-hour idle time, 4 g/L of salt and manual agitation, coincidentally the same exception as for DNT. The reason for greater recovery is unknown, but it is apparently not due to heterogeneous distribution.

Agitation method

For the most part, manual mixing is just as good as vortex mixing, but the latter gave somewhat better recoveries for RDX and 2-Am-DNT in D-49-B. Since agitation is strictly a mechanical phenomenon, it is not clear why the main effect would be significant for some but not all analytes. For RDX there is one significant interaction involving agitation: size, agitation and idle time. Figure 6 shows two bivariate plots of concentration vs size. At 30 mesh, all the results are around 0.8–0.9 μg/g. When the particle size is smaller, the results for vortex vs manual agitation diverge but only at 15 minutes of idle time. Vortexing in this case has a recovery of about 0.9 vs 0.65 for manual shaking.



a. Idle time = 0.25 hr. *b. Idle time = 4 hr.*

Figure 6. Interaction plots for RDX in Iowa 6 soil.

Table 14. Ratio of variances between levels of each factor.

	Iowa 6 soil				*Nebraska D-49-B soil*			
Analyte	*size*	*agit*	*conc*	*idle*	*size*	*agit*	*conc*	*idle*
HMX	**14.0***	0.6	0.6	0.6	0.7	1.1	2.2	**0.4**†
RDX	2.2	0.9	2.0	0.7	0.6	1.0	3.2	0.8
TNB	1.9	1.7	0.6	0.8	1.7	0.5	**6.9***	1.4
TNT	2.1	**2.5**	1.3	1.3	1.1	2.0	1.2	1.7
DNB	0.6	1.1	0.6	1.3	—	—	—	—
DNT	—	—	—	—	1.0	0.9	1.5	0.8

Ratios are: size—60 mesh/30 mesh; agitation—vortex/manual
conc—(4 g/L)/(20 g/L); idle time—15 min/4 hr
Effects in boldface are significant at 95% probability level: $F_{0.95}(15,15) = 2.4$.
Effects with * are significant at 99% probability level: $F_{0.99}(15,15) = 3.5$.
† Inverse ratio is significant.

Table 14 indicates a slight heterogeneity of variance for TNT in Iowa 6 with vortex mixing more variable than manual. Since ANOVA is robust with respect to small differences in variances, this problem may be safely disregarded. DNB in Iowa 6 soil showed a significant agitation-idle time interaction: a 15-minute idle time was better with manual mixing, but with vortexing the idle time difference was absent.

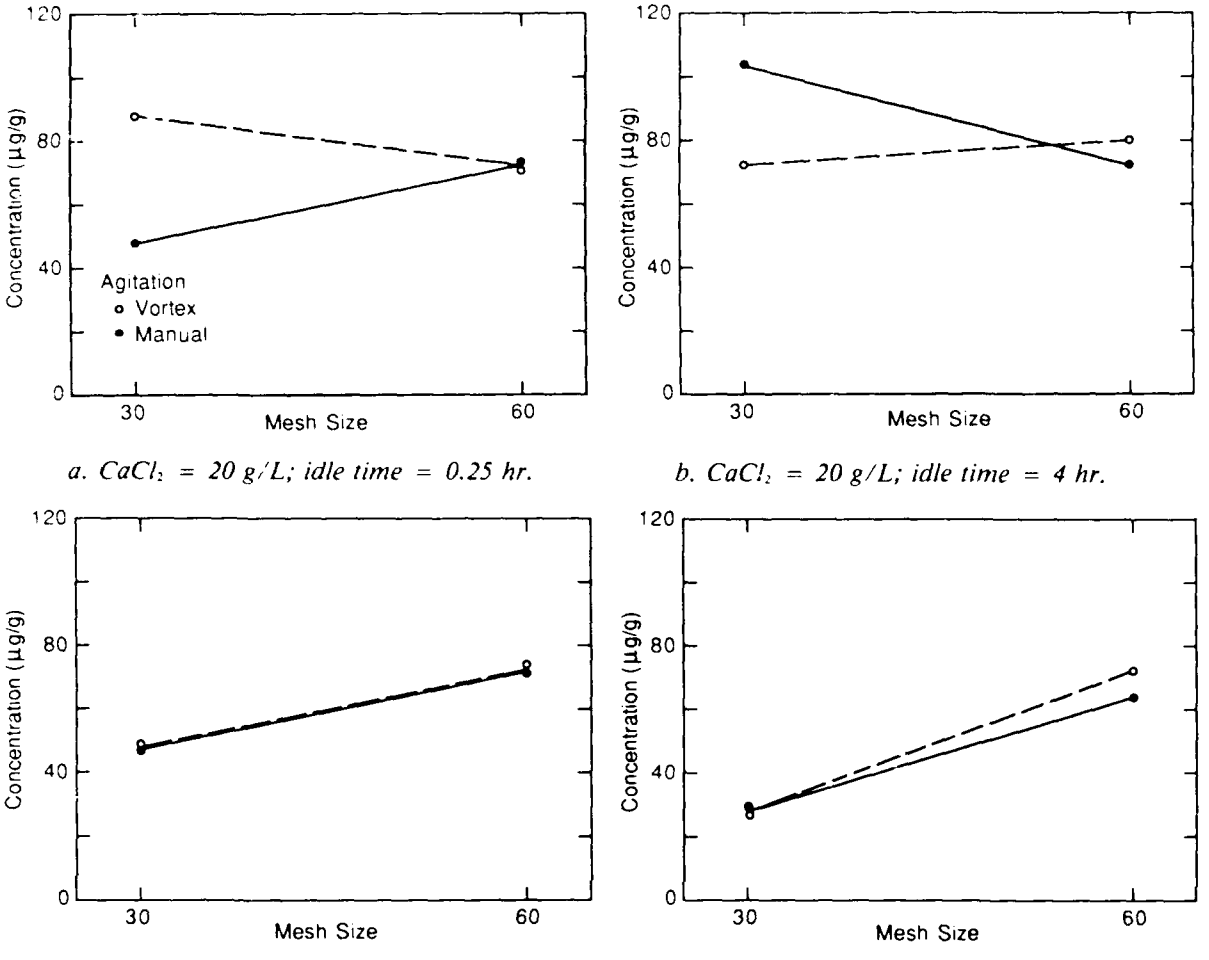
Although manual shaking is nearly equivalent to vortexing and requires no special equipment, it is not generally recommended because manual shaking styles are likely to be very different among laboratories. Uniform use of a vortex mixer at a given speed and duration would eliminate this potential source of interlaboratory variance. If a vortex mixer were unavailable, however, manual shaking would be acceptable.

Concentration of $CaCl_2$ salt solution

HMX in Iowa 6 and RDX in D-49-B were recovered more effectively at a 20-g/L salt concentration. This behavior did not extend to these analytes in the opposite soil. To understand this effect for HMX, it is necessary to consider all the significant interactions as well. Since the four-factor interaction was highly significant, its consideration will encompass also the important two- and three-factor interactions. Figure 7 contains four bivariate plots of HMX concentration vs particle size. Summary observations are

a) At 4 g/L of salt:
   1) Size is important but idle time and agitation method are not.
   2) Recoveries are always better at the smaller size.

b) At 20 g/L of salt:



a. $CaCl_2$ = 20 g/L; idle time = 0.25 hr.

b. $CaCl_2$ = 20 g/L; idle time = 4 hr.

c. $CaCl_2$ = 4 g/L; idle time = 0.25 hr.

d. $CaCl_2$ = 4 g/L; idle time = 4 hr.

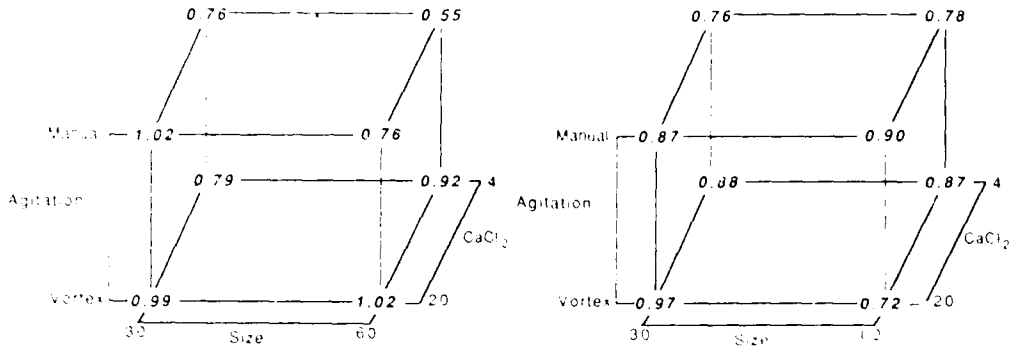
Figure 7. Interaction plots for HMX in Iowa 6 soil.

1) Vortex mixing nullifies the size effect.
2) At small size (60 mesh), recoveries are similar to those at 4 g/L regardless of agitation method.
3) Manual mixing of large particles results in recovery at short idle times similar to those at 4 g/L.
4) Manual mixing of large particles results in exceptionally high recovery at long idle times.

c) The variance at 20 g/L is slightly greater than at 4 g/L (Table 14).

Observation a indicates that the primary occurrence of the significant size effect is at low salt concentration. Observations b2 and b3 indicate that the size effect still operates for manual agitation and short idle time, but this effect is nullified by vortex mixing (b1). If the accessibility of HMX to the solvent is limited because of its depositional environment, then vortex mixing must be providing sufficient particle dispersion to overcome this. Vortex mixing is not similarly effective at 4 g/L. Since the salt solution is not added until after extraction, this implies that the small salt concentration causes reprecipitation or readsorption of the HMX so that it settles with the flocculating particles but only at 30 mesh size. This explanation is not intuitively satisfactory! Since there is a significant practical advantage to not grinding beyond , further investigation into the effect of ntration may be warranted since it is ıipulated.

ppearance of the size effect when vorte lowed by addition of 20 g/L of salt occurs a ; as well as short idle times (b1). On the other hand, for some unknown reason, manually shaken samples allowed to idle for four hours result in recoveries that exceed those of vortexing



a. Idle time = 0.25 hr. *b. Idle time = 4 hr.*

Figure 8. Cube plot for concentrations (μg/g) of RDX in Nebraska D-49-B soil.

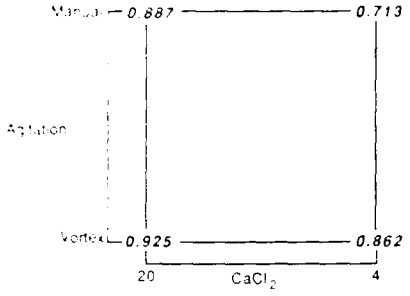


Figure 9. Square plot for concentrations at different levels of agitation and $CaCl_2$ concentrations for RDX in Nebraska D-49-B soil.

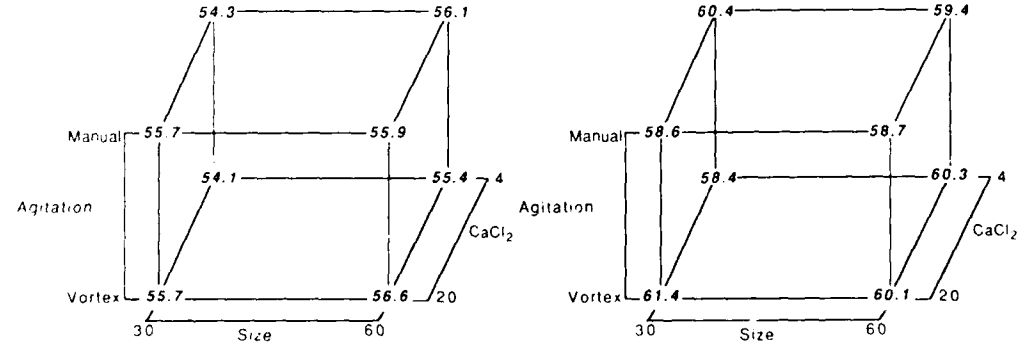
(b4). It is not an artifact of a single errant value; the replicates were 92.6 and 123.8 μg/g—two of the highest three values in the data set. We can't suggest a reasonable physical explanation other than the possibility that the highest value may be the result of an experimental error or a "hot spot" in the homogenized sample. The lower value is not out of line given that the average and standard deviation at 30 mesh are 66 and 23 μg/g, respectively.

The other soil, Nebraska D-49-B, does not show such complex behavior for HMX. Differences in soil chemistry or the lower dissolved HMX concentration could be important.

For RDX in Nebraska D-49-B soil the salt concentration effect is consistent for all comparisons (Fig. 8) but one (60 mesh, vortex, 4 hr), and there are no interactions that involve this factor. A square plot of the two main effects, agitation and salt concentration (Fig. 9), shows that the concentration effect is dominated by the contribution from manual agitation (0.17 difference) over vortex (0.06 difference). Thus the effect of concentration may be minimized when only vortex mixing is used.

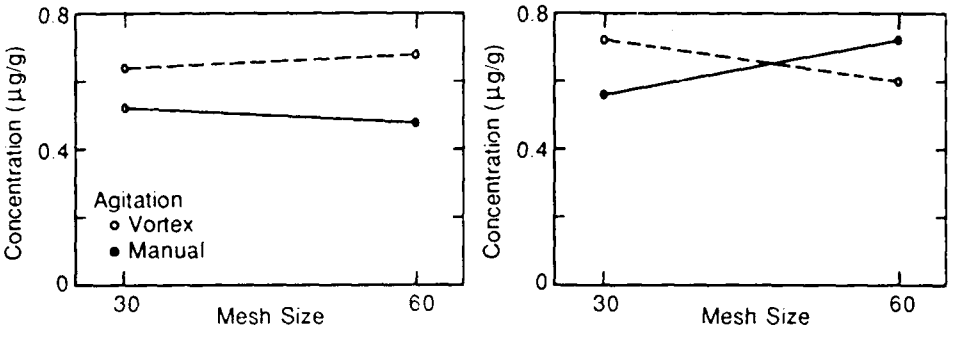
Idle time

Recoveries of TNB in Iowa 6 and 2-Am-DNT in Nebraska D-49-B benefited from a longer idle



a. Idle time = 0.25 hr. *b. Idle time = 4 hr.*

Figure 10. Cube plot of concentrations (μg/g) of TNB in Iowa 6 soil.



a. Idle time = 0.25 hr. *b. Idle time = 4 hr.*

Figure 11. Interaction plots for 2-Am-DNT in Nebraska D-49-B soil.

time. For TNB the effect was highly significant. A cube plot (Fig. 10) shows that the effect is consistent for every comparison. Although the effect was not observed for TNB in Nebraska D-49-B, the TNB variance for this soil is very heterogeneous, short times being much more variable than long times. Since this degree of heterogeneity could desensitize ANOVA tests, the data set was segregated into long and short idle times. Analysis of the resultant two 2^3 factorials indicated no significant effects after idle time had been factored out.

These observations confirm the results discussed earlier for TNB in Iowa 6. The complex forms immediately but appears to break down as the sample is allowed to stand at room temperature. The effect is specific to TNB and to Iowa 6.

For HMX in Nebraska D-49-B the $CaCl_2$ concentration and idle time interaction was significant. At four hours of idle time, concentration has little effect, but at 15 minutes, recoveries were higher at 20 g/L. For 2-Am-DNT, a highly significant three-factor interaction exists (size, agitation, and idle time). Discussion of this effect also includes those of agitation, idle time, and the interaction between them (Fig. 11). Vortexing is far superior at short idle times regardless of size but at long idle times only at 30 mesh. Hence, short idle times are advantageous at 20 g/L or with vortex mixing.

Diagnostic tests

It is necessary to establish whether hidden effects exist and whether data variances are homogeneous. Concentration was plotted against run sequence. In all cases values were distributed randomly about a horizontal line, indicating no temporal influence on results (e.g. from drift in instrumental response or in composition of standards).

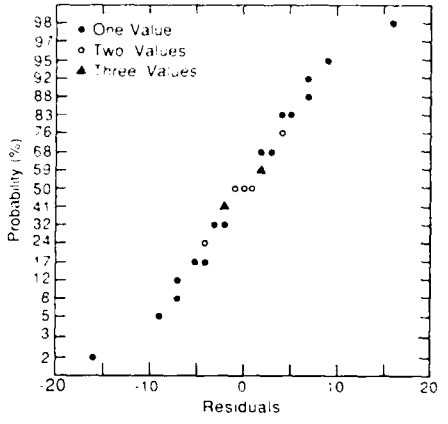


Figure 12. Probability plot of model residuals for HMX in Iowa 6 soil.

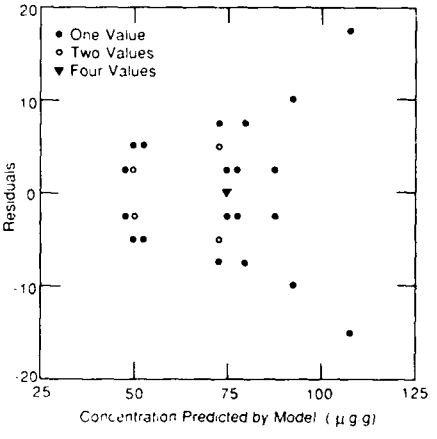


Figure 13. Model residuals as a function of HMX concentration.

Probability plots of model residuals were prepared (e.g. Fig. 12). In a few cases these were slightly nonlinear (HMX, RDX and DNB in Iowa 6; DNT in D-49-B). Model residuals were plotted against concentration predicted by the model (e.g. Fig. 13). The variances for the Iowa 6 analytes

Table 15. Standard deviations derived from ruggedness test ANOVA.

Analyte	Iowa 6	Nebraska D-49-B Heights	Nebraska D-49-B Areas	Soil recovery study
Absolute standard deviations (μg/g)				
HMX	8.3*	0.16	1.8	0.44 (1.4)†
RDX	11.8*	0.12	0.58	0.51 (1.5)†
TNB	2.2*	0.13	0.26	0.43 (1.1)†
DNB	0.14	—	—	0.13
TNT	11.7*	0.10	0.14	0.27 (22)†
DNT	—	0.08	—	0.20
Percent relative standard deviation				
HMX	11.6	7.4	35.2	—
RDX	15.7	14.1	68.7	—
TNB	3.8	7.2	12.5	—
TNT	1.6	14.4	54.4	—
DNB	25.4	—	—	—
DNT	—	15.3	—	—
2-Am-DNT	—	12.2	—	—

* Concentration of analyte outside range of homogeneous variance as determined by Jenkins and Walsh (1987). These should be compared with the values in parentheses in the last column.

† Values in parentheses are outside the range of homogeneous variance and were calculated as a percentage of the average concentration.

noted above were slightly nonuniform. This nonuniformity violates the premise of ANOVA that the variances be homogeneous, but it is not serious enough to compromise the interpretations, i.e., ANOVA is very robust. A source for DNT's nonnormality could not be found.

Precision

ANOVA was used to estimate the replication error from the 16 sets of duplicate measurements for each analyte. Table 15 lists these quantities expressed on an absolute basis and as a percentage relative to the grand average of the data set. Precisions were better than about 16% when analyte concentrations were well above the reporting limit (as in Iowa 6 for all but DNB), but precisions degraded significantly when integration was used. Peak heights offer an improvement in this case (Table 15). The ability of digital integrators to accurately locate baseline and peak maxima when signals are low is much poorer than possible by a skilled analyst.

Absolute errors may be compared with those found in recovery tests on spiked soils (Jenkins and Walsh 1987). Concentrations of analytes were generally within the range of homogeneous variance (except for RDX, TNB and TNT in Iowa 6) and may be compared with the spiked-soil data in the last column. For the three exceptions the relative variance beyond the linear range was multiplied by the grand average concentration. In most cases the spiked-soil values are comparable to those found in the ruggedness test. Only HMX and RDX in Iowa 6 exceeded these values, indicating that these analytes may exhibit larger variances in real soils than would be expected from prepared soils.

Summary

The method is quite rugged overall. Few effects were highly significant (99% probability) and no particular factor was dominant for all analytes and soil types. The recommended parameter levels for the method are vortex mixing, 15 minutes of idle time, 30-mesh particles and 10 g/L of $CaCl_2$.

Vortex mixing helps avoid the potential variability of manual shaking styles among laboratories. It sometimes damps the effects of other factors at their recommended levels (e.g. HMX in Iowa 6 at 20 g/L; DNB in Iowa 6 at 15 minutes of idle time; RDX in D-49-B). It also enhances the recovery for some analytes (RDX and 2-AmDNT in Nebraska D-49-B).

The 10-g/L concentration of $CaCl_2$ enhances the recovery for some analytes at the 20-g/L factor level (HMX in Iowa 6 and in D-49-B at short idle times; RDX in D-49-B). It eliminates the size dependence of the recovery loss for HMX in Iowa 6. The recommended level was reduced to 10 g/L based on the salting out effect at refrigerator temperatures discussed earlier.

An idle time of 15 minutes enhanced the recovery for some analytes at recommended factor levels (HMX at 20 g/L and 2-AM-DNT with vortex mixing in D-49-B). It was less satisfactory than four hours of idle time only for TNB (low recovery in Iowa 6; inflated variance in D-49-B). The low recovery for TNB is improved by allowing final solutions to sit at room temperature overnight prior to the determination of analyte concentrations.

There was no evidence for grinding-induced thermal decomposition. The 60-mesh advantage for HMX is negated by using 20 g/L of $CaCl_2$. The DNB and DNT effects are opposite and poorly understood but minor; the DNT effect may be related to organic matter.

Stability of stock standards

One major question in all analytical procedures

is how often stock standards must be replaced. To address this question we took advantage of the availability of stock standards of these explosives prepared over a period of 19 months. In all cases these stock standards were prepared by weighing out SARM-grade material, transferring it to volumetric flasks, and diluting it to volume with either methanol or acetonitrile. The stock standards were stored in a refrigerator at 4°C in the dark, and the stoppers were wrapped with Parafilm to retard solvent evaporation.

Three sets of individual stock standards were tested. The first set was prepared in methanol in August 1985. For the 1985 HMX and RDX stocks, the solution contained 40% acetonitrile to assist in initial dissolution, since these two substances dissolve very slowly in methanol. The second and third sets of standards were prepared in June 1986 and March 1987, and they were diluted to volume with acetonitrile.

In July 1987 the three sets of stock standards were compared as follows. Three replicate composite standards were prepared for each set of stock standards by adding 4.00 mL of each individual stock (3.00 mL for RDX) in a 50-mL volumetric flask (100-mL volumetric flask for the 1986 replicates) and diluting to volume with acetonitrile. Diluted working standards of each combined solution were prepared by diluting 10.00 mL to volume with acetonitrile in a 100-mL volumetric flask.

The diluted working standards were analyzed as usual using the mean integrator response of the working standard to obtain response factors for each analyte. Quantitative results for all diluted working standards were obtained using these response factors. While 2,4-DNT was not intentionally added to the 1986 standard, a small peak eluted at the proper retention time for DNT. We discovered that this impurity originated from the 1986 TNB stock standard. This impurity was also observed in the 1985 TNB stock standard at the same level relative to the response of TNB as in the 1986 stock. Both of these stock solutions were prepared from the same bottle of SARM, so it was probably due to an impurity in the solid. Since the level was the same in both 1985 and 1986 standards, it was not due to decomposition of TNB in solution. The 1987 TNB stock, on the other hand, was prepared from a different bottle of SARM, and the impurity was not observed in this stock standard.

The results of the analysis of the various diluted combined standards are presented in Appendix Table A26. The values normalized to their expected concentrations are shown in Table 16. Except for TNB in the 1986 standard and TNT in the 1985 standard, all recoveries were within 5%. The 7% low recovery for the 1986 TNB standard is understandable since it contained a known impurity that amounted to about 4% on a peak area basis, whereas the 1987 standard, on which the response factor was based, did not contain this contaminant. The 6% high recovery of TNT for the 1985 standard appears to be due to replicate a, which also showed a high value for tetryl.

Table 16. Determined concentrations of diluted combined standards normalized to expected values.*

		Normalized concentration						
Standard	*Replicate*	*HMX*	*RDX*	*TNB*	*DNB*	*Tetryl*	*TNT*	*DNT*
1987	a	1.01	1.01	1.01	1.01	1.00	1.00	1.01
	b	1.00	1.00	1.00	1.00	1.00	1.01	1.00
	c	0.99	0.99	0.99	0.99	0.99	0.98	0.99
	mean	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1986	a	0.95	0.97	0.93	0.97	1.01	0.96	—
	b	0.93	0.93	0.91	0.95	1.01	0.94	—
	c	0.98	0.99	0.96	0.99	1.07	1.00	—
	mean	0.95	0.96	0.93	0.97	1.03	0.97	—
1985	a	1.02	†	0.99	—	1.08	1.09	†
	b	0.99	†	0.96	—	1.03	1.05	†
	c	0.97	†	0.95	—	1.04	1.03	†
	mean	0.99	†	0.97	—	1.05	1.06	†

* Actual determined concentrations presented in Appendix Table A26.

† Volumes of these standards too small to allow confident use of stock.

None of the analytes showed a consistent trend toward decreasing concentrations as a function of storage time. When an analysis of variance was conducted on the data in Table 16, there were significant differences among the years for all analytes. This indicates that our ability to replicate the combination and dilution for preparing working standards from individual stock standards is better than our ability to prepare the stock standards themselves. Replicating the preparation of stock standards involves the reproducibility of the SARM from bottle to bottle as well as long-term stability of the analytical balance used to weigh out the solid.

Overall, the variation in standards prepared and stored over 23 months is minimal. We conclude that stock standards of these explosives stored in glass at 4℃ in the dark, with precautions taken to minimize solvent evaporation, can be safely used for periods up to a year. A replacement schedule of 1 year is recommended.

Stability of dilute working standard

A question remains as to how often diluted working standards need to be prepared. To test the stability of the dilute working standards, duplicate combined stock standards and duplicate dilute working standards were prepared about every five days over a 28-day period. These dilute working standards were stored over this period at 4℃ in the dark. The stoppered joints were wrapped with Parafilm to retard evaporation. Another set of duplicates was prepared at the same time as those for day 28, but they were warmed to room temperature and a small portion was removed every five days to simulate a working standard that was being used over this 28-day period. The 16 individual working standards were analyzed as a group in random order on the day following the last preparation. Response factors were obtained from the mean responses of the most recent working standard. The results are presented in Table 17. Each concentration represents a mean of two determinations.

An analysis of variance was done for each of the seven analytes (Table 17). For all the analytes except tetryl, differences were not statistically significant at the 95% confidence level, in spite of excellent agreement between duplicates, with relative standard deviations ranging from 0.52 to 1.15%.

For tetryl a statistically significant difference was observed ($F = 4.7$ compared to a table value $F_{0.95}(7,8) = 3.5$). A least-significant-difference computation indicated that only the standard stored for 24 days was significantly different from the most recent standard, while those stored 28

Table 17. Results of working standard stability study.

Days after preparation	*Concentration (μg/L)*						
	HMX	*RDX*	*TNB*	*DNB*	*Tetryl*	*TNT*	*2,4-DNT*
1	3108	3522	3189	3232	3368	3315	3225
	3132	3518	3199	3244	3294	3309	3239
6	3097	3478	3178	3206	3086	3269	3210
	3120	3501	3184	3235	3314	3346	3251
10	3091	3462	3174	3214	3055	3274	3213
	3115	3493	3192	3224	3075	3257	3204
15	3108	3448	3180	3233	3054	3273	3205
	3102	3467	3190	3102	2966	3265	3210
20	3101	3493	3161	3203	3214	3242	3203
	3120	3473	3189	3211	3355	3300	3233
24	3077	3452	3190	3202	2899*	3233	3190
	3117	3456	3196	3235	3002*	3265	3208
28	3098	3490	3185	3222	3356	3280	3233
	3107	3478	3189	3227	3205	3283	3231
28†	3061	3412	3159	3196	3186	3260	3193
	3115	3475	3217	3246	3069	3278	3228

* Significantly different from freshest standard at the 95% confidence level using a least-significant-difference test.

† Aliquot withdrawn at periods corresponding to 24, 20, 15, 10, 6 and 1 day to simulate a working standard being used over the period.

days were not significantly different. Thus the results for tetryl are inconsistent and suggest that the 24-day result was anomalous. We conclude that working standards can be prepared and used over a 28-day period if they are refrigerated and kept in the dark when not in use.

Stability of soil extracts

Another unresolved question is the stability of soil extracts. To investigate this question a series of five field-contaminated soils were extracted and processed as usual. The extracts were allowed to stand at room temperature for 24 hours and were then analyzed immediately. The extracts were also analyzed after being stored at 4 °C in the dark for 3, 6, 18, 27 and 71 days. The results are presented in Table 18.

HMX, RDX, DNB and TNT were found to be stable over the entire 71-day period in these extracts. Insufficient data were obtained for 2,4-DNT, however, to be certain of its stability, although we have no reason to suspect it to be less stable than the other analytes. Tetryl was not present in these samples so we are unable to generalize about its behavior.

It appears that the concentration of TNB in the

Table 18. Stability of soil extracts.

Storage time (days)	*Concentration (µg/L)*						
	HMX	*RDX*	*TNB*	*DNB*	*Tetryl*	*TNT*	*2,4-DNT*
				Milan 16 soil			
0	23.1	101	4.7	1.6	<d	8.3	<d
3	22.5	101	4.5	1.5	<d	8.1	<d
6	25.7	104	5.1	1.7	<d	8.7	<d
18	22.6	103	5.1	1.5	<d	8.8	<d
27	24.8	104	5.3	1.4	<d	8.1	<d
71	22.1	103	5.2	1.6	<d	8.4	<d
				Louisiana 11 soil			
0	226	676	2.1	<d	<d	13.1	<d
3	219	663	1.6	<d	<d	11.8	<d
6	239	709	2.2	<d	<d	12.7	<d
18	240	701	2.1	<d	<d	12.1	<d
27	238	706	2.2	<d	<d	11.7	<d
71	232	704	2.3	<d	<d	11.6	<d
				Iowa 6 soil			
0	55.8	67.1	78.6	0.5	<d	698	<d
3	57.0	67.7	80.9	0.4	<d	715	<d
6	56.5	66.8	84.3	0.3	<d	711	<d
18	55.1	66.5	86.5	0.4	<d	707	<d
27	55.0	68.4	86.8	0.3	<d	702	<d
71	54.6	67.0	92.6	0.5	<d	683	<d
				Nebraska D-49-A soil			
0	3.3	<d	2.1	<d	<d	<d	<d
3	2.0	<d	1.4	<d	<d	<d	<d
6	3.2	<d	2.4	<d	<d	<d	<d
18	4.6	<d	2.3	<d	<d	1.5	<d
27	4.7	<d	2.7	<d	<d	<d	<d
71	5.3	<d	2.7	<d	<d	1.3	<d
				Nebraska D-16 soil (diluted 1:10)			
0	8	<d	360	2	<d	7589	<d
3	18	<d	378	1	<d	7785	<d
6	16	<d	410	4	<d	7798	<d
18	12	<d	438	3	<d	7454	9
27	18	<d	444	5	<d	7763	9
71	<d	<d	475	5	<d	7629	11

extracts from Iowa 6 and Nebraska D-16 slowly increased over the time the extracts were held. The increase amounted to about 18% for Iowa 6 and 32% for Nebraska D-16. The increase in TNB was not accompanied by a measurable loss in the concentration of other analytes, but the small peak attributed to the TNB complex, discussed earlier, declined over storage. Thus the increase in TNB concentration was probably a result of the complex decomposing and releasing TNB during the extended storage period.

Thus it appears that extracts can be held for extended periods without adverse effect. Holding times of up to two months have been demonstrated with extracts from five field-contaminated soil samples from four states.

Initial method testing in other laboratories

The results discussed thus far and all the results described for method development reported by Jenkins and Walsh (1987) were obtained at CRREL. To assess the utility of these procedures for more general application, the method and several test samples were supplied to two other laboratories. These laboratories had no previous experience with the determination of explosive residues in soil but were acquainted with the use of RP-HPLC. Laboratory 1 supplied their own LC-18 column, while CRREL supplied the column to Laboratory 2. Two different soil samples from a group of field-contaminated soils previously characterized at CRREL were provided to each laboratory.

Laboratory 1 conducted the analyses as described by Jenkins and Walsh (1987). They added water 1:1 to the acetonitrile extracts and centrifuged prior to filtration. Laboratory 2 substituted the addition of 10 g/L of aqueous $CaCl_2$ 1:1 and allowed 15 minutes for flocculation and settling of suspended particles prior to filtration. Laboratory 2 also used peak height rather than peak area measurements for analyte determination.

The results of these analyses are presented in Table 19 along with values for the same soil obtained at CRREL (known values). For both laboratories the results compared favorably with those obtained at CRREL, particularly considering that the laboratories analyzed different subsamples of field-contaminated soil that had some inherent inhomogeneity. The method appeared to give good results with either the procedure utilizing centrifugation or the one using flocculation.

Laboratory 1 did report difficulty in getting sufficient particulate removal, even after centrifugation, to allow easy filtration prior to RP-HPLC determination. Laboratory 2, using the flocculation method, reported no difficulty at all in the filtration step. These observations reinforce our conclusion to include flocculation in the recommended method.

CONCLUSIONS AND RECOMMENDATIONS

The method of Jenkins and Walsh (1987) was tested for ruggedness, and minor modifications

Table 19. Results of method testing in two collaborating laboratories using field-contaminated soil samples supplied by CRREL.

	*Laboratory 1**				*Laboratory 2†*			
	Soil 1 conc. (μg/g)		*Soil 2 conc. (μg/g)*		*Soil 3 conc. (μg/g)*		*Soil 4 conc. (μg/g)*	
Analyte	*known***	*determined*	*known*	*determined*	*known*	*determined*	*known*	*determined*
HMX	4.2	2.1	124	117	79	98	30	25
RDX	<*d***	<*d*	1162	1120	68	93	135	149
TNB	2.0	2.6	159	170	75	62	5	5
DNB	<*d*	<*d*	<*d*	0.5	<*d*	1.3	<*d*	1.6
Tetryl	<*d*	<*d*	<*d*	<*d*	<*d*	<*d*	<*d*	<*d*
TNT	<*d*	1.0	380	375	740	718	5	8
2,4-DNT	<*d*	<*d*	4.2	3.3	<*d*	<*d*	<*d*	<*d*

* Laboratory 1 used a procedure involving addition of water 50:50 to acetonitrile extract and centrifuging prior to filtration. Quantitative results were obtained by measuring the peak area using a digitial integrator.

† Laboratory 2 used a procedure involving addition of aqueous $CaCl_2$ 50:50 to acetonitrile extract and allowing 15 min for flocculation and settling of particles prior to filtration. Quantitative results were obtained by manual peak height measurements.

** Known concentrations were obtained by analysis at CRREL. Reporting limits for these analytes are: HMX (1.6 μg/g), RDX (1.8 μg/g), TNB (1.5 μg/g), DNB (0.5 μg/g), tetryl (5.5 μg/g), TNT (0.8 μg/g), 2,4-DNT (0.8 μg/g) (Jenkins and Walsh 1987).

were made to improve its ease of use. The original procedure specified that after extraction with acetonitrile, the extract was mixed 1:1 with water, centrifuged and filtered through a 0.5-μm Millex SR filter prior to RP-HPLC determination. Experience at CRREL and a collaborating laboratory indicated that centrifugation for short periods at reasonable rpm was unable to remove sufficient particulate matter so that filtration could be conducted easily. Frequently the force required to force the liquid through the filter ruptured the filter holder, and the sample was lost.

An alternative procedure was adopted that involved adding aqueous $CaCl_2$ (10 g/L) 1:1 to the acetonitrile extract and allowing it to stand for 15 minutes prior to filtration. During the 15-minute period, flocculation and settling of the particulates occur, resulting in a solution that is easily filtered. Extensive testing with extracts from a wide variety of field-contaminated soils indicated that six of the seven analytes were unaffected by this flocculation procedure. One soil extract did demonstrate a diminished recovery of TNB, apparently due to the rapid formation of a complex of TNB with an unidentified complexing agent extracted from this soil. This TNB complex slowly decomposed when allowed to stand at room temperature for a day. No other problems were observed using this flocculation procedure, and its practical advantage when processing a full lot of 24 samples is enormous.

A number of other specific studies were also conducted, resulting in the following conclusions:

1. The combined analyte stock standard prepared from SARM* is stable for periods in excess of a year.
2. Dilute working standards prepared by diluting the combined analyte stock standard are stable for at least 28 days.
3. Soil extracts are stable for periods of at least two months when stored at 4°C in the dark.
4. The method is rugged with respect to minor variations in the specified protocol. Specifically the following were varied and found to have a negligible effect on the determined soil concentrations:
   a. particle size obtained for analysis by grinding and sieving,
   b. soil-to-solvent ratio,
   c. use of manual shaking instead of vortex mixing prior to ultrasonic bath extraction,
   d. number of samples processed simultaneously in the ultrasonic bath,
   e. concentration of $CaCl_2$ used for flocculation, and
   f. post-flocculation idle time prior to filtration.
5. Photodegradation of TNT was possible if soil samples were air-dried in direct sunlight. However, this problem is easily avoided.

A step-by-step protocol for use of this method, written in USATHAMA format (USATHAMA 1987), is presented in Appendix B.

Our experience using this method with soil samples from a wide variety of sites from five states indicates that the method is reliable and very inexpensive to use for determination of explosive residues in soil. We recommend that it be given a full collaborative test through the auspices of the AOAC to carefully define the performance characteristics attainable in everyday use.

* Standard Analytical Reference Material.

LITERATURE CITED

AOAC (1986) TNT, RDX, HMX and 2,4-DNT in wastewater and groundwater, Liquid chromatographic method. *Journal of the Association of Official Analytical Chemists,* **69:** 366–367.

Bauer, C.F., C.L. Grant and T.F. Jenkins (1986) Interlaboratory evaluation of high-performance liquid chromatographic determination of nitroorganics in munition plant wastewater. *Analytical Chemistry,* **58:** 176–182.

Box, G.E.P., W.G. Hunter and J.S. Hunter (1978) *Statistics for Experimenters.* New York: Wiley and Sons.

Cragin, J.H., D.C. Leggett, B.T. Foley and P.W. Schumacher (1985) TNT, RDX and HMX explosives in soils and sediments. Analysis techniques and drying losses. USA Cold Regions Research and Engineering Laboratory, CRREL Report 85-15.

Dyer, J.R. (1965) *Applications of Absorption Spectroscopy of Organic Compounds.* London: Prentice-Hall, p. 17.

Hubaux, A. and G. Vos (1970) Decision and detection limits for linear calibration curves. *Analytical Chemistry,* **42:** 849–855.

Jenkins, T.F. and C.L. Grant (1987) Comparison of extraction techniques for munitions residues in soil. *Analytical Chemistry,* **59:** 1326–1331.

Jenkins, T.F. and D.C. Leggett (1985) Comparison of extraction techniques and solvents for explosive residues in soil. USA Cold Regions Re-

search and Engineering Laboratory, Special Report 85-22.

Jenkins, T.F. and M.E. Walsh (1987) Development of an analytical method for explosive residues in soil. USA Cold Regions Research and Engineering Laboratory, CRREL Report 87-7.

Jenkins, T.F., C.F. Bauer, D.C. Leggett and C.L. Grant (1984) Reverse phase HPLC method for analysis of TNT, RDX, HMX and 2,4-DNT in munitions wastewater. USA Cold Regions Research and Engineering Laboratory, CRREL Report 84-29.

Jenkins, T.F., D.C. Leggett, C.L. Grant and C.F. Bauer (1986) Reversed-phase high-performance liquid chromatographic determination of nitroorganics in munitions wastewater. *Analytical Chemistry*, **58:** 170–175.

Jenkins, T.F., L.K. Knapp and M.E. Walsh (1987) Losses of explosives residues on disposable membrane filters. USA Cold Regions Research and Engineering Laboratory, Special Report 87-2.

Palazzo, A.J. and D.C. Leggett (1986a) Effect and disposition of TNT in a terrestrial plant. *Journal of Environmental Quality*, **15:** 49–52.

Palazzo, A.J. and D.C. Leggett (1986b) Effect and disposition of TNT in a terrestrial plant and validation of analytical methods. USA Cold Regions Research and Engineering Laboratory, Report 86-15.

USATHAMA (1987) U.S. Army Toxic and Hazardous Materials Agency QA Program, December 1985. Aberdeen Proving Ground, Maryland, 2nd Edition.

Walsh, M.E., L.K. Knapp and T.F. Jenkins (1988) Evaluation of disposable membrane filter units for sorptive losses and sample contamination. *Environmental Technology Letters*, **9:** 45–52.

APPENDIX A: EXPERIMENTAL DATA

Table A1. Comparison of analytical results for HMX samples flocculated with $CaCl_2$ vs those centrifuged prior to filtration.

Sample	HMX (µg/g) Centrifuged	HMX (µg/g) Flocculated	Ratio (centrifuged/floc.)
Iowa 3	1786	1926	0.93
Iowa 6	60	70	0.86
Louisiana 11	254	258	0.98
Louisiana 12	64	68	0.94
Milan 13	84	86	0.98
Milan 14	75	79	0.95
Milan 16	30	27	1.11
Milan 17	4.7	3.7	1.27
			mean = 1.00
			S.D. = 0.13

Table A2. Comparison of analytical results for RDX samples flocculated with $CaCl_2$ vs those centrifuged prior to filtration.

Sample	RDX (µg/g) Centrifuged	RDX (µg/g) Flocculated	Ratio (centrifuged/floc.)
Iowa 3	11918	12807	0.94
Iowa 6	108	115	0.94
Louisiana 11	952	972	0.98
Louisiana 12	186	185	1.01
Milan 13	470	465	1.01
Milan 14	592	616	0.96
Milan 16	137	139	0.99
Milan 17	< d	< d	--
			mean = 0.98
			S.D. = 0.03

Table A3. Comparison of analytical results for TNB samples flocculated with $CaCl_2$ vs those centrifuged prior to filtration.

Sample	TNB (µg/g) Centrifuged	TNB (µg/g) Flocculated	Ratio (centrifuged/floc.)
Iowa 3	487	468	1.04
Iowa 6	80	80	1.00
Louisiana 11	2.1	2.1	1.00
Louisiana 12	3.9	3.8	1.03
Milan 13	3.0	2.5	1.20
Milan 14	< d	< d	--
Milan 16	4.6	6.1	0.75
Milan 17	< d	< d	--
			mean = 1.00
			S.D. = 0.14

Table A4. Comparison of analytical results for TNT samples flocculated with $CaCl_2$ vs those centrifuged prior to filtration.

Sample	TNT (µg/g)		Ratio
	Centrifuged	Flocculated	(centrifuged/floc.)
Iowa 3	9249	9237	1.00
Iowa 6	686	784	0.88
Louisiana 11	13.2	14.8	0.89
Louisiana 12	15.1	12.4	1.22
Milan 13	33	35	0.94
Milan 14	1.1	1.3	0.85
Milan 16	4.1	5.5	0.75
Milan 17	1.6	1.1	1.45
			mean = 1.00
			S.D. = 0.23

Table A5. Soil-to-solvent ratio test for HMX.

Replicate	Concentration µg/g		
	2 g/50 mL	2 g/25 mL	2 g/10 mL
		Iowa 3	
1	2011	1986	1897
2	1981	2052	1987
3	1991	2047	2019
4	2031	1964	1921
5	1962	1998	2013
6	1961	1952	1972
$\bar{X}$	1990	2000	1968
S	27.7	41.7	49.5
		Louisiana 11	
1	219	224	302
2	234	224	302
3	219	218	281
4	242	226	214
5	222	225	276
6	210	250	210
$\bar{X}$	224	228	264
S	11.6	11.2	41.8

Table A6. Soil-to-solvent ratio test for RDX.

Replicate	Concentration (µg/g) 2 g/50 mL	2 g/25 mL	2 g/10 mL
	Iowa 3		
1	13585	13480	12474
2	13570	13732	12910
3	13525	13388	12644
4	14113	13383	12526
5	13332	13093	13071
6	13354	12644	12442
$\bar{X}$	13580	13287	12678
S	283	376	257
	Louisiana 11		
1	860	862	879
2	890	856	863
3	873	873	832
4	917	867	808
5	902	846	810
6	825	923	777
$\bar{X}$	878	871	828
S	32.9	27.0	37.9

Table A7. Soil-to-solvent ratio test for TNB.

Replicate	Concentration (µg/g) 2 g/50 mL	2 g/25 mL	2 g/10 mL
	Iowa 3		
1	479	471	477
2	480	480	469
3	497	491	504
4	477	466	440
5	485	479	495
6	487	488	457
$\bar{X}$	484	479	474
S	7.3	9.6	23.7
	Louisiana 11		
1	1.9	1.7	1.7
2	1.8	1.7	1.7
3	2.2	1.6	1.6
4	6.6*	1.7	1.6
5	1.3	1.9	1.6
6	1.8	1.7	1.7
$\bar{X}$	1.8	1.7	1.7
S	0.3	0.1	0.1

*An outlier using Dixon's Test and not used in statistical analysis.

Table A8. Soil-to-solvent ratio test for DNB.

Replicate	Concentration (µg/g) 2 g/50 mL	2 g/25 mL	2 g/10 mL
	Iowa 3		
1	--	38.6	38.7
2	38.9	39.4	40.4
3	40.4	39.4	41.3
4	37.1	41.3	38.3
5	37.5	37.8	38.7
6	38.0	33.4	40.1
$\bar{X}$	38.4	38.3	39.6
S	1.3	2.7	1.2
	Louisiana 11		
1	< d	< d	0.25
2	< d	< d	0.16
3	< d	< d	0.12
4	< d	< d	0.10
5	< d	< d	0.15
6	< d	< d	0.13
$\bar{X}$	--	--	0.15
S	--	--	0.05

Table A9. Soil-to-solvent ratio test for Tetryl.

Replicate	Concentration (µg/g) 2 g/50 mL	2 g/25 mL	2 g/10 mL
	Iowa 3		
1	364	455	457
2	409	419	331
3	379	368	419
4	378	451	342
5	367	366	637*
6	442	462	443
$\bar{X}$	390	420	398
S	30.1	43.8	58.2
	Louisiana 11		
1	4.3	3.4	3.7
2	6.0	4.3	3.4
3	7.3	3.3	3.0
4	3.4	3.9	3.1
5	4.1	3.3	2.6
6	3.7	3.2	3.0
$\bar{X}$	4.8	3.1	3.1
S	2.2	1.4	0.4

*An outlier using Dixon's Test and not used in statistical analysis.

Table A10. Soil-to-solvent ratio test for TNT.

Replicate	Concentration (μg/g) 2 g/50 mL	2 g/25 mL	2 g/10 mL
		Iowa 3	
1	15888	15044	13960
2	14731	14762	14084
3	14612	15326	14474
4	15019	14449	13519
5	14827	14699	14495
6	14326	14306	14406
$\bar{X}$	14901	14764	14460
S	536	376	481
		Louisiana 11	
1	11.9	12.6	12.5
2	19.6*	11.8	11.5
3	12.8	10.9	12.3
4	11.4	12.0	11.2
5	14.3	12.5	11.3
6	10.7	25.5*	11.0
$\bar{X}$	12.2	12.0	11.6
S	1.4	0.7	0.6

*An outlier using Dixon's Test and not used in statistical analysis.

Table All. Ruggedness test results (μg/g) for Iowa 6 soil.

Row	Size	Agitation*	$CaCl_2$	Idle time	Sequence	HMX	RDX	TNB	DNB	TNT
[1]	30 -	1	4 +	.25 -	[1]	46.270	66.21	53.91	.482	735.450
[2]	30 -	-1	20 -	.25 -	[2]	81.930	59.08	53.45	.555	730.670
[3]	30 -	-1	4 +	.25 -	[3]	45.960	79.29	53.44	.555	728.390
[4]	30 -	1	20 -	.25 -	[4]	56.150	101.83	53.92	.554	728.440
[5]	30 -	1	4 +	4.00 +	[5]	48.650	65.13	61.55	.366	735.090
[6]	30 -	-1	4 +	4.00 +	[6]	47.100	82.39	58.89	.609	722.010
[7]	60 +	-1	4 +	.25 -	[7]	87.060	96.50	55.45	.950	739.770
[8]	60 +	-1	20 -	.25 -	[8]	74.740	71.84	58.76	.428	737.650
[9]	60 +	-1	20 -	4.00 +	[9]	87.770	72.45	61.19	.484	731.990
[10]	30 -	1	20 -	.25 -	[10]	47.540	59.03	57.44	.524	721.540
[11]	60 +	-1	20 -	4.00 +	[11]	85.100	71.40	59.10	.862	744.160
[12]	60 +	1	4 +	.25 -	[12]	72.930	83.35	54.99	.598	738.450
[13]	60 +	1	4 +	4.00 +	[13]	75.040	80.32	58.87	.444	739.840
[14]	60 +	-1	4 +	.25 -	[14]	72.270	82.45	55.30	.607	750.182
[15]	60 +	-1	4 +	4.00 +	[15]	76.300	92.30	61.44	.930	733.670
[16]	60 +	-1	4 +	4.00 +	[16]	75.240	77.80	59.09	.589	763.470
[17]	30 -	1	20 -	4.00 +	[17]	92.610	72.71	60.50	.339	733.870
[18]	30 -	1	4 +	4.00 +	[18]	53.640	66.29	59.20	.630	749.560
[19]	30 -	-1	4 +	.25 -	[19]	56.360	58.90	54.85	.347	756.930
[20]	30 -	-1	20 -	4.00 +	[20]	69.330	63.26	57.86	.668	734.470
[21]	60 +	1	4 +	.25 -	[21]	76.950	75.97	57.25	.837	745.430
[22]	30 -	-1	20 -	.25 -	[22]	100.850	67.87	57.95	.451	760.570
[23]	30 -	-1	20 -	4.00 +	[23]	77.890	60.44	64.84	.605	744.290
[24]	60 +	1	20 -	.25 -	[24]	75.210	67.48	55.81	.590	749.920
[25]	60 +	1	20 -	.25 -	[25]	78.910	95.99	55.90	.644	736.770
[26]	60 +	-1	20 -	.25 -	[26]	74.480	77.28	54.34	.599	744.860
[27]	30 -	1	20 -	4.00 +	[27]	123.810	58.84	56.68	.147	740.410
[28]	30 -	1	4 +	.25 -	[28]	50.070	89.03	54.78	.637	744.830
[29]	60 +	1	4 +	4.00 +	[29]	67.470	71.69	59.99	.375	734.720
[30]	30 -	-1	4 +	4.00 +	[30]	52.375	87.55	57.94	.562	747.980
[31]	60 +	1	20 -	4.00 +	[31]	79.560	76.51	57.04	.641	746.250
[32]	60 +	1	20 -	4.00 +	[32]	64.860	82.33	60.41	.577	739.460

*1 = manual; -1 = vortex.

Table A12. Ruggedness test results (µg/g) for Nebraska D-49-B soil using chromatogram peak heights.

Row	Size	Agitation*	$CaCl_2$	Idle time	Sequence	HMX	RDX	TNB	TNT	2-Am-DNT	DNT
[1]	30 -	1	4 +	.25 -	9	2.402	.798	1.759	.650	.469	.635
[2]	30 -	-1	20 -	.25 -	19	2.402	1.170	1.892	.806	.578	.659
[3]	30 -	-1	4 +	.25 -	29	2.239	.780	1.666	.650	.578	.556
[4]	30 -	1	20 -	.25 -	3	2.345	1.027	2.024	.578	.000	.593
[5]	30 -	1	4 +	4.00 +	17	2.105	.844	1.808	.837	.741	.559
[6]	30 -	-1	4 +	4.00 +	14	2.117	.963	1.771	.939	.722	.624
[7]	60 +	-1	4 +	.25 -	18	1.954	1.009	1.603	.614	.664	.476
[8]	60 +	-1	20 -	.25 -	26	2.605	1.174	1.957	.924	.000	.339
[9]	60 +	-1	20 -	4.00 +	5	1.995	.734	1.750	.578	.650	.529
[10]	30 -	1	20 -	.25 -	12	2.524	1.009	1.712	.650	.578	.476
[11]	60 +	-1	20 -	4.00 +	21	1.970	.715	1.750	.693	.614	.450
[12]	60 +	1	4 +	.25 -	28	2.076	.459	1.550	.541	.433	.370
[13]	60 +	1	4 +	4.00 +	32	2.263	.771	1.868	.794	.852	.476
[14]	60 +	-1	4 +	.25 -	31	2.100	.826	1.708	.650	.722	.423
[15]	60 +	-1	4 +	4.00 +	25	2.109	.752	1.868	.845	.650	.529
[16]	60 +	-1	4 +	4.00 +	13	2.255	.981	1.771	.722	.578	.487
[17]	30 -	1	20 -	4.00 +	22	2.280	.844	1.763	.780	.433	.593
[18]	30 -	1	4 +	4.00 +	23	2.467	.679	1.691	.592	.809	.450
[19]	30 -	-1	4 +	.25 -	4	2.157	.798	1.750	.888	.736	.476
[20]	30 -	-1	20 -	4.00 +	7	2.214	.981	1.797	.715	.708	.540
[21]	60 +	1	4 +	.25 -	10	2.113	.633	1.687	.671	.582	.506
[22]	30 -	-1	20 -	.25 -	15	2.032	.804	1.645	.705	.722	.503
[23]	30 -	-1	20 -	4.00 +	2	2.109	.963	1.885	.729	.729	.582
[24]	60 +	1	20 -	.25 -	27	2.385	.780	1.792	.722	.397	.434
[25]	60 +	1	20 -	.25 -	11	2.187	.734	1.759	.657	.524	.577
[26]	60 +	-1	20 -	.25 -	16	2.043	.862	1.603	.830	.794	.476
[27]	30 -	1	20 -	4.00 +	1	2.182	.899	1.847	.751	.404	.688
[28]	30 -	1	4 +	.25 -	24	2.076	.725	1.371	.671	.498	.582
[29]	60 +	1	4 +	4.00 +	20	2.280	.798	1.839	.715	.664	.635
[30]	30 -	-1	4 +	4.00 +	8	2.157	.789	1.813	.606	.736	.423
[31]	60 +	1	20 -	4.00 +	6	2.035	1.000	1.700	.758	.765	.556
[32]	60 +	1	20 -	4.00 +	30	2.019	.807	1.725	.650	.650	.524

*1 = manual; -1 = vortex.

Table A13. Ruggedness test results (μg/g) for Nebraska D-49-B soil using chromatogram peak areas.

Row	Size	Agitation*	$CaCl_2$	Idle time	Sequence	HMX	RDX	DNB	TNT
[1]	30 -	1	4 +	.25 -	9	3.400	.384	1.827	.267
[2]	30 -	-1	20 -	.25 -	19	8.634	.779	2.135	.548
[3]	30 -	-1	4 +	.25 -	29	3.472	.320	2.070	.284
[4]	30 -	1	20 -	.25 -	3	6.079	.972	1.889	.404
[5]	30 -	1	4 +	4.00 +	17	2.197	1.268	1.968	.437
[6]	30 -	-1	4 +	4.00 +	14	5.336	.943	1.978	.295
[7]	60 +	-1	4 +	.25 -	18	3.634	1.097	2.270	.000
[8]	60 +	-1	20 -	.25 -	26	7.915	.836	1.920	.280
[9]	60 +	-1	20 -	4.00 +	5	6.424	.790	2.494	.241
[10]	30 -	1	20 -	.25 -	12	4.046	.730	1.744	.366
[11]	60 +	-1	20 -	4.00 +	21	6.403	.795	1.894	.290
[12]	60 +	1	4 +	.25 -	28	2.323	.435	1.757	.295
[13]	60 +	1	4 +	4.00 +	32	1.923	.329	2.242	.612
[14]	60 +	-1	4 +	.25 -	31	1.964	.559	2.032	.000
[15]	60 +	-1	4 +	4.00 +	25	3.244	.906	1.968	.354
[16]	60 +	-1	4 +	4.00 +	13	3.361	1.020	1.856	.276
[17]	30 -	1	20 -	4.00 +	22	2.197	.860	1.933	.145
[18]	30 -	1	4 +	4.00 +	23	5.781	.491	1.749	.000
[19]	30 -	-1	4 +	.25 -	4	2.331	.948	2.274	.214
[20]	30 -	-1	20 -	4.00 +	7	6.754	1.047	2.070	.406
[21]	60 +	1	4 +	.25 -	10	3.307	.724	2.160	.505
[22]	30 -	-1	20 -	.25 -	15	6.388	.870	2.006	.211
[23]	30 -	-1	20 -	4.00 +	2	5.799	.889	2.183	.205
[24]	60 +	1	20 -	.25 -	27	5.872	.940	2.206	.000
[25]	60 +	1	20 -	.25 -	11	8.505	.332	2.856	.000
[26]	60 +	-1	20 -	.25 -	16	4.445	.980	2.068	.498
[27]	30 -	1	20 -	4.00 +	1	5.651	.472	2.136	.463
[28]	30 -	1	4 +	.25 -	24	6.664	1.046	2.251	.148
[29]	60 +	1	4 +	4.00 +	20	5.211	.348	1.896	.465
[30]	30 -	-1	4 +	4.00 +	8	3.291	1.372	2.101	.410
[31]	60 +	1	20 -	4.00 +	6	10.266	3.312	2.758	.195
[32]	60 +	1	20 -	4.00 +	30	6.480	.432	1.962	.000

*1 = manual; -1 = vortex.

Table A14. Analysis of variance for HMX in Iowa 6 soil.

	Effect	Std error effect	t-Ratio	
Average	71.07	1.46	48.68	
size (s)	10.84	2.92	3.71	**
agitation (a)	-3.44	2.92	-1.18	
CaCl2 (C)	-16.69	2.92	-5.72	**
idletime (i)	4.95	2.92	1.69	
sa	-1.81	2.92	-.62	
sC	14.52	2.92	4.97	**
si	-5.10	2.92	-1.75	
aC	.74	2.92	.25	
ai	7.76	2.92	2.66	*
Ci	-6.44	2.92	-2.21	*
saC	-.11	2.92	-.04	
sai	-11.87	2.92	-4.07	**
sCi	2.81	2.92	.96	
aCi	-6.61	2.92	-2.26	*
saCi	10.83	2.92	3.71	**

*significant at 95%

**significant at 99%

Table A15. Analysis of variance for RDX in Iowa 6 soil.

	Effect	Std error effect	t-Ratio
Average	75.42	2.09	36.03
size (s)	8.61	4.19	2.06
agitation (a)	.74	4.19	.18
CaCl2 (C)	6.05	4.19	1.45
idletime (i)	-3.17	4.19	-.76
sa	-1.79	4.19	-.43
sC	-.41	4.19	-.10
si	-.09	4.19	-.02
aC	-8.14	4.19	-1.94
ai	-4.97	4.19	-1.19
Ci	2.14	4.19	.51
saC	-.24	4.19	-.06
sai	5.24	4.19	1.25
sCi	-2.92	4.19	-.70
aCi	-1.79	4.19	-.43
saCi	1.90	4.19	.45

Table A16. Analysis of variance for TNB in Iowa 6 soil.

	Effect	Std error effect	t-Ratio	
Average	57.567	.386	149.269	
size (s)	.483	.771	.626	
agitation (a)	-.353	.771	-.458	
CaCl2 (C)	-.516	.771	-.669	
idletime (i)	4.191	.771	5.433	**
sa	-.198	.771	-.257	
sC	.494	.771	.641	
si	-.524	.771	-.680	
aC	.871	.771	1.129	
ai	-.411	.771	-.532	
Ci	.434	.771	.563	
saC	-.364	.771	-.472	
sai	-.166	.771	-.215	
sCi	-.001	.771	-.001	
aCi	.456	.771	.591	
saCi	-.669	.771	-.868	

**significant at 99%

Table A17. Analysis of variance for TNT in Iowa 6 soil.

	Effect	Std error effect	t-Ratio
Average	740.35	2.06	358.89
size (s)	3.88	4.13	.94
agitation (a)	-3.19	4.13	-.77
CaCl2 (C)	2.53	4.13	.61
idletime (i)	-.54	4.13	-.13
sa	1.33	4.13	.32
sC	-.72	4.13	-.17
si	-.65	4.13	-.16
aC	.81	4.13	.20
ai	2.83	4.13	.69
Ci	-1.10	4.13	-.27
saC	-6.11	4.13	-1.48
sai	-4.23	4.13	-1.02
sCi	1.75	4.13	.42
aCi	-2.44	4.13	-.59
saCi	-.30	4.13	-.07

Table A18. Analysis of variance for DNB in Iowa 6 soil.

	Effect	Std error effect	t-Ratio	
Average	.5683	.0255	22.2728	
size (s)	.1328	.0510	2.6013	*
agitation (a)	-.0885	.0510	-1.7342	
CaCl2 (C)	.0531	.0510	1.0410	
idletime (i)	-.0331	.0510	-.6491	
sa	-.0044	.0510	-.0857	
sC	.0100	.0510	.1960	
si	-.0108	.0510	-.2107	
aC	-.0090	.0510	-.1764	
ai	-.1353	.0510	-2.6503	*
Ci	-.0304	.0510	-.5952	
saC	-.1036	.0510	-2.0306	
sai	.0211	.0510	.4140	
sCi	-.0893	.0510	-1.7489	
aCi	.0140	.0510	.2743	
saCi	-.0444	.0510	-.8696	

*significant at 95%

Table A19. Analysis of variance for HMX in D-49-B soil.

	Effect	Std error effect	t-Ratio	
Average	2.1937	.0286	76.8003	
size (s)	-.0887	.0571	-1.5525	
agitation (a)	.0801	.0571	1.4015	
CaCl2 (C)	-.0286	.0571	-.5000	
idletime (i)	-.0677	.0571	-1.1849	
sa	-.0392	.0571	-.6860	
sC	.0174	.0571	.3052	
si	.0006	.0571	.0098	
aC	.0067	.0571	.1171	
ai	.0081	.0571	.1411	
Ci	.1472	.0571	2.5765	*
saC	.0309	.0571	.5416	
sai	.0181	.0571	.3162	
sCi	.0859	.0571	1.5043	
aCi	.0244	.0571	.4278	
saCi	-.0396	.0571	-.6925	

*significant at 95%

Table A20. Analysis of variance for RDX in D-49-B soil.

	Effect	Std error effect	t-Ratio	
Average	.8471	.0211	40.1969	
size (s)	-.0649	.0421	-1.5392	
agitation (a)	-.0934	.0421	-2.2154	*
CaCl2 (C)	-.1186	.0421	-2.8144	*
idletime (i)	-.0042	.0421	-.1008	
sa	-.0405	.0421	-.9609	
sC	.0465	.0421	1.1032	
si	.0144	.0421	.3411	
aC	-.0555	.0421	-1.3168	
ai	.0639	.0421	1.5155	
Ci	.0729	.0421	1.7290	
saC	-.0374	.0421	-.8867	
sai	.1185	.0421	2.8115	*
sCi	.0108	.0421	.2551	
aCi	-.0133	.0421	-.3144	
saCi	-.0244	.0421	-.5783	

*significant at 95%

Table A21. Analysis of variance for TNB in D-49-B soil.

	Effect	Std error effect	t-Ratio
Average	1.7539	.0224	78.3378
size (s)	-.0165	.0448	-.3685
agitation (a)	-.0209	.0448	-.4662
CaCl2 (C)	-.0674	.0448	-1.5047
idletime (i)	.0730	.0448	1.6303
sa	.0096	.0448	.2150
sC	.0496	.0448	1.1083
si	.0035	.0448	.0782
aC	-.0263	.0448	-.5862
ai	.0004	.0448	.0084
Ci	.0939	.0448	2.0965
saC	.0360	.0448	.8040
sai	.0091	.0448	.2038
sCi	.0291	.0448	.6504
aCi	.0425	.0448	.9491
saCi	-.0165	.0448	-.3685

Table A22. Analysis of variance for TNT in D-49-B soil.

	Effect	Std error effect	t-Ratio
Average	.7160	.0177	40.3793
size (s)	-.0114	.0355	-.3225
agitation (a)	-.0548	.0355	-1.5457
CaCl2 (C)	-.0088	.0355	-.2485
idletime (i)	.0311	.0355	.8759
sa	.0113	.0355	.3190
sC	-.0237	.0355	-.6680
si	-.0128	.0355	-.3613
aC	-.0006	.0355	-.0159
ai	.0611	.0355	1.7219
Ci	.0583	.0355	1.6444
saC	.0166	.0355	.4670
sai	.0022	.0355	.0617
sCi	.0734	.0355	2.0709
aCi	-.0492	.0355	-1.3870
saCi	-.0156	.0355	-.4388

Table A23. Analysis of variance for DNT in D-49-B soil.

	Effect	Std error effect	t-Ratio
Average	.5931	.0330	17.9834
size (s)	.0061	.0660	.0929
agitation (a)	-.0864	.0660	-1.3094
CaCl2 (C)	.1180	.0660	1.7889
idletime (i)	.1519	.0660	2.3024*
sa	.1108	.0660	1.6790
sC	-.0241	.0660	-.3657
si	.0115	.0660	.1743
aC	.0441	.0660	.6689
ai	.0778	.0660	1.1787
Ci	-.0181	.0660	-.2748
saC	-.0893	.0660	-1.3530
sai	.0076	.0660	.1156
sCi	-.0595	.0660	-.9020
aCi	.0595	.0660	.9020
saCi	.0199	.0660	.3013

*significant at 95%

Table A24. Analysis of variance for 2-AM-DNT in D-49-B soil.

	Effect	Std error effect	t-Ratio
Average	.5227	.0142	36.9291
size (s)	-.0720	.0283	-2.5435*
agitation (a)	.0364	.0283	1.2850
CaCl2 (C)	-.0195	.0283	-.6889
idletime (i)	.0353	.0283	1.2453
sa	.0097	.0283	.3444
sC	.0216	.0283	.7639
si	.0379	.0283	1.3380
aC	-.0090	.0283	-.3179
ai	.0033	.0283	.1148
Ci	-.0154	.0283	-.5431
saC	-.0191	.0283	-.6756
sai	-.0004	.0283	-.0132
sCi	.0303	.0283	1.0686
aCi	-.0164	.0283	-.5785
saCi	.0430	.0283	1.5190

*significant at 95%

Table A25. Analysis of variance for 2-AM-DNT in D-49-B soil.

	Effect	Std error effect	t-Ratio
Average	.6302	.0136	46.2675
size (s)	.0061	.0272	.2248
agitation (a)	-.0864	.0272	-3.1707**
CaCl2 (C)	.0439	.0272	1.6101
idletime (i)	.0777	.0272	2.8537 *
sa	.0366	.0272	1.3440
sC	-.0241	.0272	-.8856
si	.0115	.0272	.4222
aC	.0441	.0272	1.6198
ai	.0778	.0272	2.8541 *
Ci	.0560	.0272	2.0562
saC	-.0151	.0272	-.5548
sai	.0818	.0272	3.0014**
sCi	-.0595	.0272	-2.1842 *
aCi	.0595	.0272	2.1842 *
saCi	-.0543	.0272	-1.9919

*significant at 95%

Table A26. Results of tests on long-term stability of stock standards.

Standard		Concentration (µg/L) HMX	RDX	TNB	DNB	Tetryl	TNT	2,4-DNT
1987	a	3142	2659	3216	3266	3333	3324	3258
	b	3108	2638	3196	3231	3347	3330	3222
	c	3093	2604	3147	3193	3303	3261	3196
known value		3120	2640	3194	3238	3331	3312	3232
1986	a	3841	3096	3634	4069	4280	3932	--
	b	3757	2972	3540	3971	4281	3841	--
	c	3974	3152	3728	4154	4507	4058	--
known value		4048	3180	3888	4176	4224	4076	--
1985	a	3881	*	3557	--	3940	3631	*
	b	3754	*	3448	--	3786	3514	*
	c	3732	*	3436	--	3670	3477	*
known value		3792	2458	3597	--	3661	3341	1248

* Volume of remaining stock solution to small to allow confident use of this standard.

APPENDIX B: METHOD DOCUMENTATION IN USATHAMA (1987) FORMAT

Method No. SM02

Reversed-Phase HPLC Method for the Determination of Explosive Residues in Soil

I. SUMMARY

A. ANALYTES. The following analytes can be determined using this method: HMX, RDX, 135TNB, 13DNB, tetryl, 246TNT, 24DNT.

B. MATRIX. This method is suitable for determination of explosive residues in soil and sediment.

C. GENERAL METHOD. This method involves extraction of soil using acetonitrile in an ultrasonic bath followed by determination using reversed-phase HPLC - UV 254 nm.

II. APPLICATION

A. TESTED CONCENTRATION RANGE. For a 2-g soil sample extracted with 50 mL of acetonitrile in which a 100-μL aliquot is injected, this method was found to be linear over the following concentration ranges: HMX (5.0–101 μg/g), RDX (0.5–212 μg/g), 135TNB (0.5–97 μg/g), 13DNB (0.3–104 μg/g), tetryl (5.3–105 μg/g), 246TNT (0.5–51.0 μg/g) and 24DNT (0.4–15.6 μg/g). Linear range can be extended by the use of smaller injection volumes.

B. SENSITIVITY. The response of the UV detector at 254 nm for HMX, RDX, 135TNB, 13DNB, tetryl, 246TNT and 24DNT was estimated at 5.28×10^{-4}, 4.91×10^{-4}, 7.80×10^{-4}, 3.20×10^{-4}, 1.54×10^{-3}, 3.05×10^{-4} and 4.35×10^{-4} absorbance units, respectively, at the certified reporting limits given below.

C. REPORTING LIMITS. Certified reporting limits (CRLs) for the following analytes were determined over a four-day period using the method of Hubaux and Vos. Reporting limits were calculated to be: HMX (1.6 μg/g), RDX (1.8 μg/g), 135TNB (1.5 μg/g), 13DNB (0.5 μg/g), tetryl (5.5 μg/g), 246TNT (0.8 μg/g) and 24DNT (0.8 μg/g), using a 100-μL injection volume of 50% of the acetonitrile soil extract and 50% of 10 g/L aqueous $CaCl_2$.

D. INTERFERENCES. In one soil a small loss of TNB was observed due to formation of a complex with an unknown soil component when flocculated with $CaCl_2$ prior to filtration. In no other cases were interferences for any other analyte observed. A second column was found to be useful for confirming analyte identity. Chromatographic peaks have been observed for HMX and tetryl on the primary analytical column, but they were not confirmed on the secondary column.

E. ANALYSIS RATE. Approximately 24 samples can be extracted and analyzed over a two-day period if stock solutions have been prepared in advance.

F. SAFETY INFORMATION. The normal safety precautions appropriate to use of flammable organic solvents should be employed.

III. APPARATUS AND CHEMICALS

A. GLASSWARE/HARDWARE

1. Injection syringe—Hamilton, liquid syringe, 500 μL
2. Filters—0.5-μm Millex SR, disposable
3. Pipettes—10 mL and 50 mL volumetric, glass
4. Scintillation vials—20 mL, glass
5. Disposable syringes—Plastipak, 10 mL
6. Test tubes—2.5 cm $\times$ 20 cm, screw cap, Teflon-lined caps
7. Volumetric flasks—25, 50, 100, 200, 250 and 500 mL

B. INSTRUMENTATION

1. HPLC—Perkin Elmer Series 3 (or equivalent) equipped with a 100-μL sample loop injector and a fixed-wavelength 254-nm UV detector. A flow rate of 1.5 mL/min of 50% methanol and 50% water is used with both RP columns.
2. Strip chart recorder.
3. Digital integrator—HP 3390 (or equivalent)
4. Vortex mixer
5. Ultrasonic bath
6. LC-18 (Supelco) RP-HPLC column, 25 cm $\times$ 4.6 mm (5 μm)
7. LC-CN (Supelco) RP-HPLC column, 25 cm $\times$ 4.6 mm (5 μm)

C. ANALYTES

1. HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine),
   boiling point—decomposes,
   melting point—282°C,
   solubility in water at 22.5°C—5.0 mg/L,
   octanol/water partition coefficient—1.3,
   CAS #2691-41-0
2. RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine),
   boiling point—decomposes,
   melting point—203.5°C,
   solubility in water at 25°C—60 mg/L,
   octanol/water partition coefficient—7.5,
   CAS #121-82-4
3. 135TNB (1,3,5-trinitrobenzene),
   boiling point—decomposes,
   melting point—122°C,
   octanol/water partition coefficient—15,
   CAS #25377-32-6
4. 13DNB (1,3-dinitrobenzene),
   boiling point—302°C,
   melting point—90°C,
   octanol/water partition coefficient—31
   CAS #99-65-01
5. Tetryl (methyl-2,4,6-trinitrophenylnitramine),
   boiling point—187°C (explodes),

melting point—131℃,
octanol/water partition coefficient—43
CAS #479-45-8

6. 246TNT (2,4,6-trinitrotoluene),
boiling point—280℃ (explodes),
melting point—80.1℃,
solubility in water—100 mg/L,
octanol/water partition coefficient—68,
CAS #118-96-7
7. 24DNT (2,4-dinitrotoluene),
boiling point—300℃ (decomposes),
melting point—70°,
solubility in water—300 mg/L,
octanol/water partition coefficient—95,
CAS #121-14-2

D. REAGENTS AND SARMS

1. HMX—SARM quality
2. RDX—SARM quality
3. 135TNB—SARM quality
4. 13DNB—SARM quality
5. Tetryl—SARM quality
6. 246TNT—SARM quality
7. 24DNT—SARM quality
8. Methanol—HPLC grade
9. Acetonitrile—HPLC grade
10. Water—Reagent grade
11. $CaCl_2$—Reagent grade, solution 10 g/L.

IV. CALIBRATION

A. INITIAL CALIBRATION

1. Preparation of Standards. SARM for each analyte was dried to constant weight in a vacuum desiccator in the dark. About 0.1 g of each dried SARM was weighed out to the nearest 0.1 mg and transferred to individual 100-mL volumetric flasks and diluted to volume with acetonitrile. Stock standards are stored in a refrigerator at 4℃ in the dark. Stock standards are usable for periods up to a year after the date of preparation.

   A combined analyte stock standard is prepared by combining 10.0 mL of the HMX, RDX and tetryl stock standards and 5.00 mL of 135TNB, 13DNB, 246 TNT and 24DNT stock standards in a 100-mL volumetric flask and bringing to volume with aceotonitrile. This solution contains about 50 mg/L of 135TNB, 13DNB, 246TNT and 24DNT and 100 mg/L of HMX, RDX and tetryl.

A series of working standards were prepared by diluting this combined stock standard with methanol as shown below:

CALIBRATION STANDARDS

Standard	Aliquot (mL)	Size of volumetric flask (mL)	Solution conc.* (μg/L) 246TNT, 135TNB, 13DNB, 24DNT	Solution conc.* (μg/L) HMX, RDX, Tetryl
A	10 †	25	20,000	40,000
B	10 †	100	5,000	10,000
C	10 **	25	2,000	4,000
D	10 **	50	1,000	2,000
E	10 **	100	500	1,000
F	10 **	250	200	400
G	5 **	250	100	200
H	5 **	500	50	100
I	1 **	250	20	40
J	1 **	500	10	20
K	0.5**	500	5	10

* Concentrations correspond to 100% extraction with 50 mL of solvent.
† Aliquot of combined stock.
** Aliquot of Standard B.

2. Instrument Calibration. All standards are diluted 50:50 with water before injecting. Duplicate injections of each standard over the concentration range of interest are sequentially injected in the HPLC in random order. Peak areas or peak heights are obtained for each analyte. The retention order under the specified conditions is HMX (2.6 min), RDX (3.8 min), 135TNB (5.2 min), 13DNB (6.3 min), tetryl (7.0 min), 246 TNT (8.5 min) and 24DNT (10.2 min).

3. Analysis of Calibration Data. The acceptability of a linear model with zero intercept is assessed using the protocol specified in the USATHAMA QA Program (2nd Edition, March 1987). Experience indicates that a linear model with zero intercept is appropriate. Thus the slope of the best-fit regression line is equivalent to a response factor which can be compared with values obtained from replicate analyses of a single standard each day.

B. DAILY CALIBRATION. Standard B, described above, is used for daily calibration after diluting 50:50 with water. Standard B can be used for a period of 28 days after preparation. It is analyzed in duplicate at the beginning of the day and singly after the last sample of the day. Response factors for each analyte are obtained from the mean peak areas or peak heights obtained over the course of the day and compared with the response factor obtained for initial calibration. These values must agree within $\pm 2S$, or a new initial calibration must be obtained.

V. CERTIFICATION TESTING

A. PREPARATION OF SPIKING SOLUTIONS. Individual analyte spiking solutions are prepared in an identical manner to that described for the cal-

ibration stocks. A combined analyte spiking standard is prepared by adding 25 mL of the 135TNB, 13DNB, 246TNT and 24DNT stocks and 50 mL of the HMX, RDX and tetryl stocks solutions to a 500-mL volumetric flask and bringing to volume with acetonitrile. A series of spiking standards are prepared as described below:

SPIKING SOLUTIONS

Aliquot of combined analyte spiking standard (mL)	Capacity of volumetric flask (mL)	Solution conc. (μg/mL)		Soil conc. (μg/g)*	
		246TNT, 135TNB 24DNT, 13DNB	HMX, RDX, Tetryl	246TNT, 135TNB 24DNT, 13DNB	HMX, RDX, Tetryl
stock	no dilution	50	100	25	50
25	50	25	50	12.5	25
20	100	10	20	5	10
10	100	5	10	2.5	5
5	100	2.5	5	1.25	2.5
2	100	1	2	0.50	1.0
1	100	0.5	1	0.25	0.5
1	200	0.25	0.5	0.12	0.25

* Assuming 1 mL spiking solution added to 2 g of soil.

B. PREPARATION OF CONTROL SPIKES. Spiked soil samples are prepared by placing a series of 2.00-g subsamples of USATHAMA Standard Soil in individual 2.5-cm × 20-cm glass test tubes. Each tube was spiked by addition of 1.00 mL of one of the spiking standards described above and allowed to equilibrate for 1 hr prior to addition of the extraction solvent.

C. ANALYSIS OF SOIL SPIKES. Soil spikes are processed and analyzed as described below for real samples.

VI. SAMPLE HANDLING AND STORAGE

A. SAMPLING PROCEDURE. Soil samples are refrigerated in the dark as soon as feasible after collection.

B. CONTAINERS. All containers used to store wet or dried soil should be cleaned according to procedures specified in the USATHAMA QA Manual.

C. STORAGE CONDITIONS. All soil samples are stored in a refrigerator at 4°C in the dark until extracted. Samples should be processed as soon as possible after receipt and always within seven days after receipt.

D. HOLDING TIME LIMITS. Soil samples must be refrigerated in the dark until processed. Soils should be dried and extracted within seven days of receipt.

E. SOIL DRYING/HOMOGENIZATION.* Soil samples are air-dried to constant weight prior to extraction. Care is taken to ensure that the soil is not exposed to direct sunlight during the drying period.

Dried soil is ground to pass a 30-mesh sieve and homogenized thoroughly on a roller mill or by manual shaking in a closed container.

VII. PROCEDURE

A. EXTRACTION/DILUTION WITH AQUEOUS $CaCl_2$. A 2-g subsample of each dried soil is placed in individual 2.5-cm × 20-cm screw-cap glass test tubes. A 50-mL aliquot of acetonitrile is added to each tube, the tubes capped, the suspensions subjected to vortex mixing for 1 minute, and the tubes placed in an ultrasonic bath for 18 hours.

The samples are removed from the sonic bath and allowed to cool and settle for 30 minutes. A 10-mL aliquot of the supernatant is removed and combined with a 10-mL aliquot of aqueous $CaCl_2$ solution (10 g/L) in a glass scintillation vial. The vials are shaken and allowed to stand for 15 minutes. A 10-mL portion of the supernatant is placed in a Plastipak syringe and filtered through a 0.5-μm Millex SR filter membrane. The first several mL are discarded and the remainder retained for analysis. The samples are then allowed to stand at room temperature in the dark overnight.†

B. DETERMINATION. Determination of analyte concentrations in the diluted extracts is obtained by RP-HPLC on a fixed-wavelength 254-nm UV detector. A 100-μL loop is overfilled by injecting 500 L of the acetonitrile/$CaCl_2$ solution through the loop and injecting onto an LC-18 column eluted with 1.5 mL/min of 50/50 methanol-water. Retention times and capacity factors for the seven analytes of interest and a number of potential interferences are given in Table 1 for both LC-18, the primary analytical column, and LC-CN, the confirmation column. A chromatogram obtained for the seven primary analytes is shown in Figure 1.

VIII. CALCULATIONS

A. RESPONSE FACTORS. Since a linear calibration curve with zero intercept is to be expected, the results on a daily basis are calculated using response factors calculated for each analyte. The mean response ($\overline{R}$) for each analyte from repeated determination of STANDARD B is obtained in either peak area or peak height units. The response factor for each analyte (RF) is then obtained by dividing the mean response by the known solution concentration (C) in units of μg/L:

$$RF = \overline{R}/C. \tag{1}$$

B. ANALYTE CONCENTRATIONS. Solution concentrations (μg/L) in the extracts (C_a) are then obtained by dividing the response obtained for each analyte (R_a) by the appropriate response factor (RF_a):

$$C_a = \frac{R_a}{RF_a}. \tag{2}$$

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* Soil drying is preferable to enable good sample homogenization prior to subsampling. Experience indicates the method works with undried samples as well.

† This period of standing at room temperature prior to determination was found to improve 135TNB recovery for some soils.

TABLE 1. Retention times and capacity factors for primary analytes and potential interferences on LC-18 and LC-CN columns eluted with 50:50 water-methanol at 1.5 mL/min.

	Retention time (min)		Capacity factor* k	
Substance	LC-18	LC-CN	LC-18	LC-CN
HMX	2.55	9.87	0.49	3.94
RDX	3.82	6.56	1.23	2.28
135TNB	5.16	4.27	2.02	1.14
13DNB	6.25	4.27	2.65	1.14
Tetryl	7.04	8.08	3.12	3.04
246TNT	8.47	5.11	3.95	1.56
24DNT	10.15	4.94	4.94	1.47
Benzene	11.50	3.35	5.76	0.79
SEX	2.27	5.25	0.33	1.63
TAX	2.68	3.70	0.57	0.85
2A46DT	9.10	5.86	4.32	1.93
4A26DNT	8.88	5.48	4.19	1.74
24DANT	2.79	3.36	0.63	0.68
26DANT	2.56	3.36	0.50	0.68
26DNT	9.88	4.73	4.78	1.37
245TNT	8.47	6.34	3.95	2.17
Toluene	23.39	†	12.8	†
Nitrobenzene	7.38	3.83	3.32	0.92
m-Nitrotoluene	14.78	†	7.64	†
Cyclohexanone	3.94	2.75	1.30	0.38

* Capacity factors are based on an unretained peak for nitrate at 1.71 min on LC-18 and 2.00 min on LC-CN.
† No data.

Concentration in soil (X_a), on a $\mu g/g$ basis, is then obtained by multiplying the solution concentrations by the volume of extraction solvent (0.050 L) and dividing by the actual mass of dry soil extracted (M):

$$X_a = \frac{C_a(0.050)}{M} \quad (3)$$

IX. DAILY QUALITY CONTROL

A. CONTROL SPIKES. Spiked soil samples are prepared as described for Class 1 methods in the USATHAMA QA Program (2nd Edition, March 1987). This requires the use of a method blank, a single spike at two times the certi fied reporting limit, and duplicate spikes at ten times the certified reporting limit for each analytical lot. Control spikes are prepared using the appropriate spiking solution in an identical manner as described in section V.

B. CONTROL CHARTS. The control charts required are described for Class 1 methods in USATHAMA QA Program (2nd Edition, March 1987). This will require use of standard Shewhart $\bar{X}$ and R charts for the duplicate high spikes and moving average $\bar{X}$ and R charts for the single low spike. Details on the charting procedures required are specified in USATHAMA QA Program (2nd Edition, March 1987).

X. REFERENCES

Hubaux, A. and G. Vos (1970) Decision and detection limit for linear calibration curves. *Analytical Chemistry,* **42:** 849-855.

Jenkins, T.F. and M.E. Walsh (1987) Development of an analytical method for explosive residues in soil. USA Cold Regions Research and Engineering Laboratory, CRREL Report 87-7.

Jenkins, T.F., P.W. Schumacher, M.E. Walsh and C.F. Bauer (1987) Development of an analytical method for explosive residues in soil. Part 2. Additional development and ruggedness testing.

USATHAMA (1987) USATHAMA QA Program, December 1985 (2nd Edition, March 1987). USA Toxic and Hazardous Materials Agency, Aberdeen Proving Ground, MD, 21010-5401.

A facsimile catalog card in Library of Congress MARC format is reproduced below.

Jenkins, Thomas F.

Development of an analytical method for the determination of explosive residues in soil. Part II: Additional development and ruggedness testing / by Thomas F. Jenkins, Patricia W. Schumacher, Marianne E. Walsh and Christopher F. Bauer. Hanover, N.H.: U.S. Army Cold Regions Research and Engineering Laboratory; Springfield, Va.: available from National Technical Information Service, 1988.

vi, 57 p., illus.; 28 cm. (CRREL Report 88-8.)

Bibliography: p. 25.

1. Chemical analysis. 2. Explosives. 3. Soils. 4. Soil contamination. I. Schumacher, Patricia W. II. Walsh, Marianne E. III. Bauer, Christopher F. IV. United States Army. Corps of Engineers. V. Cold Regions Research and Engineering Laboratory. VI. Series: CRREL Report 88-8.

☆ U. S. GOVERNMENT PRINTING OFFICE: 1988--500-050--82017